THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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VOLUME 89
AUGUST TO DECEMBER, 1945

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to The Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $1.75. Subscription per volume (three issues), $4.50.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Massachusetts, between July 1 and October 1, and to the Department of Zoology, Washington University, St. Louis, Missouri, during the remainder of the year.

Entered as second-class matter May 17, 1930, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

LANCASTER PRESS, INC., LANCASTER, PA.

CONTENTS

No. 1. August, 1945

PAGE

Annual Report of the Marine Biological Laboratory 1

Whiting, Anna
Dominant lethality and correlated chromosome effects in Habrobracon eggs x-rayed in diplotene and in late metaphase I 61

Harvey, Ethel Browne
Stratification and breaking of the Arbacia punctulata egg when centrifuged in single salt solutions 72

Pace, D. M.
The effect of cyanide on respiration in Paramecium caudatum and Paramecium aurelia 76

Metz, Charles B.
The agglutination of starfish sperm by fertilizin 84

Kozloff, Eugene N.
Cochliophilus depressus gen. nov., sp. nov. and Cochliophilus minor sp. nov., holotrichous ciliates from the mantle cavity of Phytia setifer (Cooper) 95

Scheer, Bradley T.
The development of marine fouling communities 103

Spiegelman, S. and Florence Moog
A comparison of the effects of cyanide and azide on the development of frogs' eggs 122

No. 2. October, 1945

Kidder, George W. and Virginia C. Dewey
Studies on the biochemistry of Tetrahymena. IV. Amino acids and their relation to the biosynthesis of thiamine 131

LeFevre, Paul G.
Certain chemical factors influencing artificial activation of Nereis eggs. 144

Hibbard, Hope and George I. Lavin
A study of the Golgi apparatus in chicken gizzard epithelium by means of the quartz microscope 157

Hayashi, Teru
Dilution medium and survival of the spermatozoa of Arbacia punctulata. I. Effect of the medium on fertilizing power 162

Kozloff, Eugene N.
Heterocineta phoronopsidis sp. nov., a ciliate from the tentacles of Phoronopsis viridis Hilton 180

Abstracts of Scientific Papers Presented at the Marine Biological Laboratory, Summer of 1945 184

No. 3. December, 1945

Tyler, Albert and Bradley T. Scheer
Natural heteroagglutinins in the serum of the spiny lobster, Panulirus interruptus. II. Chemical and antigenic relation to blood proteins.... 193
Rogick, Mary Dora
Studies on marine Bryozoa. I. Aeverrillia setigera (Hincks) 1887..... 201
Rogick, Mary Dora
Studies on fresh-water Bryozoa. XVI. Fredericella australiensis var. browni, n. var..... 215
Kidder, George W. and Virginia C. Dewey
Studies on the biochemistry of Tetrahymena. VII. Riboflavin, pantothen, biotin, niacin and pyridoxine in the growth of T. geleii W..... 229
Ris, Hans
The structure of meiotic chromosomes in the grasshopper and its bearing on the nature of "chromomeres" and "lamp-brush chromosomes"..... 242

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AUG 2 1 1945

Volume 89 Number 1

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SERIAL LIST

A SERIAL list of the holdings of The Marine Biological Laboratory has been published as a separately bound supplement to The Biological Bulletin. This supplement lists with cross references the titles of journals in the Library; additional titles and changes are published annually. A few extra copies of the original list are still available. Orders may be directed to The Marine Biological Laboratory.

MICROFILM SERVICE

THE Library of The Marine Biological Laboratory is now prepared to supply microfilms of material from periodicals included in its extensive list. Through the generosity of Dr. Athertone Seidell, the essential equipment has been set up and put into operation. The Staff of The Marine Biological Laboratory Library is anxious to extend the Microfilm Service, particularly at this time when distance makes the Library somewhat inaccessible to many who normally use it. Investigators who wish films should send to the Librarian the name of the author of the paper, its title, and the name of the periodical in which it is printed, together with the volume and year of publication. The rates are as follows: $.30 for papers up to 25 pages, and $.10 for each additional 10 pages or fraction thereof. It is hoped that many investigators will avail themselves of this service.

Vol. 89, No. 1 August, 1945

THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

FORTY-SEVENTH REPORT, FOR THE YEAR 1944—FIFTY-SEVENTH YEAR

I. TRUSTEES AND EXECUTIVE COMMITTEE (AS OF AUGUST 8, 1944) 1
STANDING COMMITTEES
II. ACT OF INCORPORATION 3
III. BY-LAWS OF THE CORPORATION 4
IV. REPORT OF THE TREASURER 5
V. REPORT OF THE LIBRARIAN 10
VI. REPORT OF THE DIRECTOR 11
Statement 11
Addenda:
1. Memorial to Dr. Caswell Grave 14
2. The Report of the Committee of Review 15
3. The Decennial Review Submitted to the Committee of Review 17
4. The Staff 38
5. Investigators and Students 40
6. Tabular View of Attendance, 1940–1944 46
7. Subscribing and Co-operating Institutions 46
8. Evening Lectures 47
9. Shorter Scientific Papers 47
10. Members of the Corporation 49

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such

case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Massachusetts, at 11:30 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Mass., at 10 A.M. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years; and in addition there shall be two groups of Trustees as follows:

(B) Trustees ex officio, who shall be the President and Vice President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer, and the Clerk;

(C) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next Annual Meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee *Emeritus* for life. The Trustees *ex officio* and *Emeritus* shall have all the rights of the Trustees except that Trustees *Emeritus* shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

IX. The Trustees shall have the control and management of the affairs of the Corporation; they shall elect a President of the Corporation who shall also be Chairman of the Board of Trustees; and shall also elect a Vice President of the Corporation who shall also be the Vice Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

X. Any person interested in the Laboratory may be elected by the Trustees to a group to be known as Associates of the Marine Biological Laboratory.

XI. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

XII. The account of the Treasurer shall be audited annually by a certified public accountant.

XIII. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

IV. THE REPORT OF THE TREASURER

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY:

Gentlemen:

Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1944.

The accounts have been audited by Messrs. Seamans, Stetson, and Tuttle, certified public accountants. A copy of their report is on file at the Laboratory and inspection of it by members of the Corporation will be welcomed.

The principal summaries of their report—The Balance Sheet, Statement of Income and Expense, and Current Surplus Account—are appended hereto as Exhibits A, B and C.

The following are some general statements and observations based on the detailed reports:

I. Assets

1. Endowment Assets

As of December 31, 1944, the total book value of all the Endowment Assets, including the Scholarship Funds, was $983,900.57, a loss for the year of $19,808.06.

The Scholarship Funds were increased by the gift of $5,000.00 from Bishop James E. Cassidy of Fall River to establish the "Reverend Arsenious Boyer Burse." The principal losses incurred were due, as in the previous year, to the foreclosure of mortgage participations on New York City realty and the subsequent sale of the properties. In 1944 a four story tenement at 4856 Broadway on which the Laboratory held a mortgage investment of $30,057.10 was sold, the Laboratory receiving $6,026.00 cash and a new mortgage participation for $17,000, and sustaining a loss of $7,031.10. The property at 47 Murray Street, a five story loft building, was sold entirely for cash, at a loss of $9,569.24 on an investment of $21,928.75.

At the end of the year $803,403.76 was invested in marketable securities (bonds, preferred stocks and common stocks) with a market value of $825,005.80. $163,769.79 was invested in mortgage participations on New York City real estate and in real estate participations resulting from mortgage foreclosures. $16,727.02 was in uninvested principal cash.

The Treasurer's estimate of the actual value of the $163,769.79 in mortgage and real estate participations held on December 31 is $87,750.00. With the market value of $825,005.80 on marketable securities and the $16,727.02 in cash this makes a total current valuation of $929,482.82 compared with total book value of $983,900.57 and original capital value of $1,116,924.25.

2. Plant Assets

The total of Plant Assets (excluding the Gansett and Devil's Lane tracts) was $1,333,726.48 after deduction of $656,341.78 accumulated Depreciation Reserve, a decrease for the year of $7,699.40. Depreciation charges for 1944 were $26,929.31. The Reserve Fund was increased to a total of $16,895.62 by $3,529.41 transferred from current income (representing $279.41 profit on sale of Gansett lots, the Crane Company dividends, and part of the dividends on the General Biological Supply House stocks) and $93.99 interest received from the temporary investment of $10,000 of the Reserve Fund in U.S.A. Treasury bonds.

3. Current Assets

Current Assets including cash, inventories, and investments not in the Endowment Funds at cost, amounted to $202,239.67, an increase of $8,127.98. Current Liabilities totalled $2,181.09. The special reserve fund for repairs and replacements, made up of a portion of the 1943 income from the United States Navy rentals, and the value of certain equipment received from the Navy in lieu of restoration and repairs upon termination of the Navy lease, was $15,998.62 at the end of the year. Current Surplus was $184,059.96, $4,442.14 under the total for 1943.

II. Income and Expenditures

Total Income was $164,240.13, an increase of $4,943.19 over the 1943 income. Total Expenses were higher, $160,013.13, including Depreciation Reserves of $26,929.31 and special hurricane damage repairs of $2,466.17, but there was an actual net surplus of $4,227.00 for the year.

This surplus compares favorably with the $19,323.67 surplus in 1943 which resulted largely from the $20,150.00 rental from the United States Navy combined with reduced expenditures, and the deficit of $17,211.93 for 1942. Some of the reductions in 1944 income were a decline of $3,600.91 in endowment income, a

loss of $6,000 in net income from the Supply Department compared with 1943, and a reduction of $2,286.00 in the dividends from the General Biological Supply House. The principal gains were Mrs. W. Murray Crane's gift of Otis Elevator stock valued at $2,325.00, and an increase of over $4,000 in net income from Research.

The income and expense items, although more normal than in 1943, still do not reflect what may be regarded as regular operations. Expenditures for equipment and necessary improvements, for example, are still unavoidably under what they should be to maintain the Laboratory at full efficiency. Some reserves have been built up for a few of these expenditures, but the Laboratory needs a larger endowment income to take care of maintenance.

EXHIBIT A

MARINE BIOLOGICAL LABORATORY BALANCE SHEET, DECEMBER 31, 1944

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee		$ 968,737.59	
Securities and Cash in Minor Funds		15,162.98	
			$ 983,900.57
Plant Assets:			
Land	$ 111,425.38		
Buildings	1,327,675.21		
Equipment	186,122.42		
Library	329,639.23		
	$1,954,862.24		
Less Reserve for Depreciation	656,341.78	$1,298,520.46	
Reserve Fund, Securities and Cash		16,895.62	
Book Fund, Securities and Cash		18,310.40	
			$1,333,726.48
Current Assets:			
Cash		$ 27,513.52	
Accounts Receivable		12,357.71	
Inventories:			
Supply Department	$ 43,964.75		
Biological Bulletin	19,498.15	63,462.90	
Investments:			
Devil's Lane Property	$ 46,260.84		
Gansett Property	1,900.42		
Stock in General Biological Supply House, Inc.	12,700.00		
Other Investment Stocks	20,095.00		
Retirement Fund	12,966.30	93,922.56	
Prepaid Insurance		4,184.40	
Items in Suspense		798.58	
			$ 202,239.67
Total Assets			$2,519,866.72

Liabilities

Endowment Funds:			
Endowment Funds	$ 967,113.46		
Reserve for Amortization of Bond Premiums	1,624.13		
		$ 968,737.59	
Minor Funds		15,162.98	
			$ 983,900.57
Plant Funds:			
Donations and Gifts		$1,172,564.04	
Other Investments in Plant from Gifts and Current Funds		161,162.44	
			$1,333,726.48
Current Liabilities and Surplus:			
Accounts Payable		$ 2,181.09	
Reserve for Repairs and Replacements		15,998.62	
Current Surplus (Exhibit C)		184,059.96	
			$ 202,239.67
Total Liabilities			$2,519,866.72

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1944

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 27,291.16		$ 27,291.16
Library Fund		5,718.91		5,718.91
Donations		2,325.00		2,325.00
Instruction	$ 8,485.47	5,570.00	$ 2,915.47	
Research	4,981.44	13,654.38		8,672.94
Evening Lectures	45.85		45.85	
Biological Bulletin and Membership Dues	4,102.15	7,952.96		3,850.81
Supply Department	37,307.27	45,588.92		8,281.65
Mess	20,225.08	17,878.72	2,346.36	
Dormitories	30,761.47	13,190.82	17,570.65	
(Interest and Depreciation charged to above 3 Departments)	25,076.43			25,076.43
Dividends, General Biological Supply House, Inc.		16,510.00		16,510.00
Dividends, Other Investment Stocks		785.00		785.00
Rents:				
Bar Neck Property	759.46	4,800.00		4,040.54
Janitor House	21.35	360.00		338.65
Danchakoff Cottages	278.44	643.33		364.89
Rooms in Laboratory, Special		420.00		420.00
Sale of Library Duplicates and Micro Film		194.90		194.90
Microscope and Apparatus Rental		1,168.24		1,168.24
Sundry Income		187.79		187.79

Maintenance of Plant:				
Buildings and Grounds	18,759.11		18,759.11	
Apparatus Department	3,765.15		3,765.15	
Chemical Department	1,681.50		1,681.50	
Library Expense	6,756.08		6,756.08	
Workmen's Compensation Insurance	440.09		440.09	
Truck Expense	327.35		327.35	
Bay Shore Property	93.41		93.41	
Great Cedar Swamp	20.25		20.25	
General Expenses:				
Administration Expense	15,275.38		15,275.38	
Endowment Fund Trustee and Safe-Keeping	1,015.28		1,015.28	
Bad Debts	592.50		592.50	
Special Repairs on account of 1944 Hurricane Damage	2,466.17		2,466.17	
Reserve for Depreciation	26,929.31		26,929.31	
	$160,013.13	$164,240.13	$100,999.91	$105,226.91
Excess of Income over Expense carried to Current Surplus	4,227.00		4,227.00	
	$164,240.13		$105,226.91	

EXHIBIT C

MARINE BIOLOGICAL LABORATORY, CURRENT SURPLUS ACCOUNT, YEAR ENDED DECEMBER 31, 1944

Balance January 1, 1944			$188,502.10
Add:			
Excess of Income over Expense		$ 4,227.00	
Gain on Gansett Lots Sold		176.04	
Bad Debts Recovered		37.64	
Reserve for Depreciation charged to Plant Funds		26,929.31	31,369.99
			$219,872.09
Deduct:			
Payments from Current Funds during Year for Plant Assets:			
Buildings	$ 3,064.00		
Equipment	1,542.52		
Library	5,559.12		
	$10,165.64		
Less Received for Plant Assets Sold	172.00		
		$ 9,993.64	
Pensions Paid	$ 3,460.00		
Less:			
Retirement Fund Income $223.07			
Retirement Fund Gain on Securities 351.86			
Retirement Fund, Recovery on account of 1943 loss .51	575.44		
		$ 2,884.56	

Transfers to Reserve Fund:			
Portion of Dividends from General Biological Supply House, Inc.	$ 2,500.00		
Dividends from Crane Company	750.00		
Profit on Gansett Lots for 1943	279.41		
		$ 3,529.41	
Building Fixtures and Equipment Received from First Naval District, transferred to Plant Funds.	$ 7,225.00		
Less Loss on Fixtures and Equipment Discarded	620.48		
		$ 6,604.52	
Repairs and Replacements Made by First Naval District during their occupancy of properties, set up as a Reserve		12,800.00	
			35,812.13
Balance, December 31, 1944			$184,059.96

Respectfully submitted,
DONALD M. BRODIE,
Treasurer

V. REPORT OF THE LIBRARIAN

The sum of $11,239.77 appropriated to the library in 1944 was expended as follows: books, $760.49; serials, $2,626.99; binding, $884.00; express, $60.14; supplies, $416.17; salaries, $6,239.77; back sets, $214.50; insurance, $50.00; sundries, $2.21; total, $11,254.27. The cash earnings of the library reverting to the laboratory were $194.90: from sale of duplicates, $38.73; microfilms, $144.86; serials lists, $11.31.

Of the Carnegie Corporation of New York Fund, $2,433.69 was expended for the completion of five and partial completion of nine back sets and two books.

The sum appropriated by the Woods Hole Oceanographic Institution for 1944 was $1,900.00. A balance of $263.08 remaining from 1943 made an available total of $2,163.08. Of this sum $113.69 was expended on current books and journals and $1,100.00 on salaries, leaving a balance of $949.39. A comparison of the amount spent on current books, journals and back sets during the pre-war years with that of the war years will show that this accumulating budget balance will be expended when the material for which it was designated shall have become available.

During 1944 the library received 678 current journals: 248 (10 new) by subscription to the Marine Biological Laboratory; 15 (none new) to the Woods Hole Oceanographic Institution; exchanges 201 (three new) with the "Biological Bulletin" and 23 (one new) with the Woods Hole Oceanographic Institution publications; 186 as gifts to the former and five to the latter. The Marine Biological Laboratory acquired 169 books: 119 by purchase of the Marine Biological Laboratory; six by purchase of the Woods Hole Oceanographic Institution; nine gifts from the authors, 22 from the publishers and 13 from miscellaneous donors. There were 18 back sets of serial publications completed: ten purchased by the Marine Biological Laboratory (five with the "Carnegie Fund"); two secured by exchange with the "Biological Bulletin"; one by exchange with the Woods Hole Oceanographic Institution publications; and five by duplicate material exchange and by

gift. Partially completed sets were 59: purchased by the Marine Biological Laboratory, 23 (nine with "Carnegie Fund"); by exchange with the "Biological Bulletin," one; and by exchange of duplicate material and by gift, 35. In addition, 15 of the odd journal numbers presented by Dr. Dorothy R. Stewart (126 in all) were fitted into gaps in our sets.

The reprint additions to the library number 2,404: current of 1943, 401; current of 1944, 58; and of previous dates, 1,945. A total of 3,957 reprints, 1,321 not duplicates of our holdings, were presented to the library: 1,378 by Mrs. G. N. Calkins; 2,306 by Dr. Dorothy R. Stewart; 192 by Dr. Libbie H. Hyman; and 81 by Dr. D. A. Fraser.

It is with great pleasure that two very valuable gifts are acknowledged as presented to the library this year. Dr. Walter E. Garrey has presented his collection of reprints to be incorporated in the library's reprint holdings. As yet no count of these has been made. More detailed acknowledgment will occur in a later report. The same delayed account will be given of the reprints from Dr. E. B. Meigs' library, a gift of Mrs. Meigs. In addition to the reprints, Mrs. Meigs included in her gift long runs of fourteen different journals. As a further gift from Mrs. Meigs three of these sets will be bound and, with an appropriate book plate inserted, will be substituted for the old volumes now in the library.

At the end of the year 1944 the library contained 52,885 bound volumes and 133,054 reprints.

VI. THE REPORT OF THE DIRECTOR

To the Trustees of The Marine Biological Laboratory:

Gentlemen:

I beg to submit the following report of the fifty-seventh session of the Marine Biological Laboratory for the year 1944.

During the year the Laboratory has coped with difficulties brought on by the war—shortage of labor, and materials much needed for research and for the maintenance of the plant and the Supply Department—and with a hurricane which fortunately did not seriously damage our buildings. The immediate problem now reflects the encouraging change in the general situation throughout the country; it is to find laboratory space for the large number of investigators who expect to return in 1945.

1. *Attendance*

The anticipated increase in the number of investigators and students indicates that we have already passed the low point in the curve of attendance. This can be seen in the chart on page 20 which forms a part of the report prepared for the Committee of Review. Our numbers in the five year period from 1936–1940 were the greatest in the history of the Laboratory; in 1942 they decreased by nearly 50 per cent. The year 1943 showed a still further decline. A definite improvement is seen in the record of 1944, as shown in the Tabular View of Attendance on page 21. The prospects for a still larger number in 1945 are excellent.

From the curves on the chart, one can see the effect on attendance of changing conditions in the Laboratory and in the country at large. When new buildings

were erected here, the attendance increased sharply. The first World War reversed the upward trend, but only for one year. The business depression, felt from 1932–1935, affected chiefly the number of "New Investigators." The present war has reduced our attendance to the level of 25 years ago. After 1945 there should be a rapid rise, but with our present buildings and equipment we cannot accommodate more investigators and students than we had in 1940.

New Investigators are those who come here for the first time; after the first year they are classified among "Returning Investigators." They are chiefly "Investigators under Instruction," that is, graduate students and Fellows. Over a long period of years they have constituted nearly one third of all investigators in attendance. Recently this proportion, as shown in the Tabular View of Attendance, has grown smaller; but actually, until the war, the number of graduate students, Fellows, and young instructors present each summer did not diminish. What happened was that many came as Research Assistants, probably for economic reasons.

Since 1941 the number of beginning investigators has declined by about 75 per cent from its previous level. The loss of so large a proportion of this important source of new members and future supporters of the Laboratory will be felt for many years. We fervently hope that those who are now prevented from working here will eventually return to us.

It is a pleasure to report that the number of institutions represented during the war years has not greatly diminished, and that the list of supporting institutions receives new additions every season.

2. *Laboratory Activities*

During the summer all of the usual activities of the Laboratory were carried on. After a lapse of one year, the weekly seminars were resumed, nine being held. In addition to these, several small groups of investigators met to discuss topics in which all were especially interested. It was the general opinion that more meetings of this kind should be held. All of the courses of instruction were given, the total registration of students being 75, a moderate increase over the preceding year. Dr. John B. Buck, who served for two seasons with signal success as head of the Invertebrate Zoology course, resigned at the end of the summer. The Committee on Instruction accepted his resignation with regret, and selected Dr. Frank A. Brown, of Northwestern University, to succeed him as instructor in charge.

3. *Associates*

The Trustees, at the regular meeting this year, directed the Executive Committee to appoint a committee to consider the advisability of establishing a new kind of membership in the Corporation, to which those interested in the welfare of the Laboratory might be elected. Mrs. W. Murray Crane, Mr. Lawrence Saunders, Dr. J. P. Warbasse (all of whom were elected to membership in the Corporation at the August meeting), Dr. G. H. A. Clowes, and the Director, were asked to discuss the matter. This committee felt that there are many people without special training in Biology, who have a sincere interest in the Laboratory, and that such friends would appreciate a formal connection with it. Following the Committee's approval

of the plan, a special meeting of the Trustees was held in New York on December 9, 1944 to amend the By-laws so that this new type of membership could be made possible. The amendment which was adopted reads as follows: "Any person interested in the Laboratory may be elected by the Trustees to a group known as 'Associates of the Marine Biological Laboratory.'" It is hoped that both summer and permanent residents of Woods Hole and the vicinity may become members, and that friends in other parts of the country may also join.

At this special meeting the opinion was voiced by several Trustees that a winter meeting should be held regularly in order that current Laboratory problems could be discussed.

4. *The Committee of Review*

When the Friendship Fund in 1924 contributed a large sum to the Laboratory for endowment purposes, the Trustee of the endowment was directed to call once every ten years upon a Committee of Review to make a study of the work of the Laboratory. This Committee, which consists of nine members, includes a representative of the National Academy of Sciences, of the National Research Council, of the American Association for the Advancement of Science, and one professor of Biology from each of the following universities: Chicago, Columbia, Harvard, Pennsylvania, Princeton, and Yale. Its function is to determine whether the Laboratory continues to perform valuable services in biological research. The complete text of the Deed of Trust, in which the duties of the Committee are set forth, is printed in the 26th Annual Report which appears in Vol. 47 of the "Biological Bulletin."

The Committee first met in 1934 and voted that the Laboratory was satisfactorily fulfilling the purpose for which the endowment was given. The second decennial Committee met this year. Its findings, and the statement of the President and Director of the Laboratory regarding our activities during the years 1934–1943 are appended to this report. The Committee, in addition to its formal vote of approval, pointed out that in order to maintain a high level of usefulness, the Laboratory should secure additional funds for endowment and for purposes which are specified in their report. These recommendations, coming from a group of biologists, the majority of whom were not connected with the administration of the Laboratory, should be given most careful consideration.

5. *The Hurricane*

The September hurricane did not seriously damage our buildings. No water came in, as happened in the 1938 storm, but roofs and windows suffered. Some of the slate from the Dormitory and Apartment House roofs was blown off, many pieces imbedding themselves in distant houses. Fortunately no one was struck by these flying missiles. The Cayadetta wharf was practically demolished, and the sea wall badly broken by the tremendous waves that tossed great stones on to the street. The wharf has been partially restored by the Oceanographic Institution which has used it for the past two years. Had the full fury of the storm struck at high tide we might well have sustained a loss, due to sea water, even greater than that which we suffered in 1938. The wind, whose velocity far exceeded that

of the previous hurricane, levelled a great number of trees in the Gansett and Devil's Lane tracts, on Dr. Clowes' property, and in the Fay Woods.

Against the destructive power of winds we can do little, but it is possible to protect the Brick Building from high water. The matter of increased protection should be given consideration.

6. *Loss by Death*

This year the Corporation has lost by death Prof. William Trelease who was elected in 1888 at the first regular meeting of the Trustees after the incorporation of the Laboratory.

7. *Gift*

The Laboratory acknowledges with sincere appreciation the receipt of 100 shares of Otis Elevator stock valued at $2,325.00, a gift of Mrs. W. Murray Crane.

8. *Election of Trustee*

At the meeting of the Corporation held August 8, 1944, L. G. Barth, Associate Professor of Zoology at Columbia University, was elected to fill the vacancy caused by the resignation of Prof. I. F. Lewis.

9. There are appended as parts of this report:

1. Memorial to Dr. Caswell Grave.
2. The Report of the Committee of Review.
3. The Decennial Review—Submitted to the Committee of Review.
4. The Staff.
5. Investigators and Students.
6. Tabular View of Attendance, 1940–1944.
7. Subscribing and Co-operating Institutions.
8. Evening Lectures.
9. Shorter Scientific Papers.
10. Members of the Corporation.

Respectfully submitted,
CHARLES PACKARD,
Director

1. MEMORIAL TO DR. CASWELL GRAVE

By Prof. R. A. Budington

It is with the greatest reluctance, and with true sorrow, that today we must include among those permanently lost to the Corporation the name of Caswell Grave. Those who knew him, as most of us here did, will miss his genial personality, with his habit of industry, his steady, keen interest in everything biological, his strict integrity of character; and the Board of Trustees will be very conscious of the absence of his sincere interest in the ongoing of the Laboratory, its policies, and its scientific significance.

Grave was born a Hoosier, on a farm in Monrovia, Indiana, and was very nearly 74 years of age at the time of his death at his home in Winter Park, Florida, last January 8th. He graduated from Earlham College in 1895, with Phi Beta Kappa rank; his alma mater honored him with her Doctor of Laws degree in 1928. His graduate studies were done at Johns Hopkins University, which conferred the Ph.D. in 1899. Meanwhile, he had spent summers at the Fisheries Bureau in Woods Hole, and at the Johns Hopkins Laboratory in Jamaica. After two further years of study as Bruce Fellow, he was appointed to the Hopkins teaching staff, a relation he continued for 18 years, for 13 of which he held the rank of Associate Professor. In 1919 he was appointed to the headship of the Zoological Department at Washington University, St. Louis, where the new Rebstock Laboratory had just been built. Here he gathered about him a staff of men of outstanding competency, and put the department on a basis widely recognized for scholarship and general efficiency.

Other responsibilities carried by Grave were: Director of the U. S. Fisheries Laboratory, Beaufort, N. C., 1902–1906; Shellfish Commissioner of Maryland, 1906–1912. In World War I he was ranked a captain in the Chemical Warfare Service. He was an active member of the AAAS; the American Society of Naturalists; a member of Sigma Xi; by turn he was Secretary-Treasurer, Vice President, and President of the American Society of Zoologists. As for the Marine Biological Laboratory, he was an outstandingly successful director of the Invertebrate Course from 1912–1917; a Trustee for 20 years, 1920–1940; thereafter, Trustee Emeritus. Few, if any, have taken the welfare of the Laboratory more seriously to heart than did he.

Grave's research interest embraced three quite different fields: pelecypod mollusca as to structure, physiology, and life histories; echinoderms, with special reference to embryology, and intraphyla relationships; while in later years he attacked the problem of metamorphosis in the ascidians, with special reference to the chemical factors retarding or accelerating it.

It is not too much to say that Caswell Grave was a wise man; and in the truest sense, in all that the appellations should imply, he was a "gentleman and a scholar." We are glad to pause and offer him such honor as we may, today.

August 9, 1944

2. Minutes of the Committee of Review of the Marine Biological Laboratory

The Committee of Review provided for in the Deed of Trust Covering Funds for Endowment, Friendship Fund, Inc., and Central Hanover Bank and Trust Company of New York, met at the Marine Biological Laboratory, Woods Hole, Massachusetts, on August 9, 1944, at 9:00 A.M.

Mr. Lawrason Riggs, President of the Corporation, read the Call of Meeting, and commented on the history of the origin of the Deed of Trust, and on the duties of the Committee.

Present:

Professor W. C. Allee—representing The University of Chicago
Professor G. A. Baitsell—representing Yale University

Professor A. F. Blakeslee—representing The American Association for the Advancement of Science
Professor A. B. Dawson—representing Harvard University
Professor W. K. Gregory—representing The National Academy of Science
Professor R. W. Griggs—representing The National Research Council
Professor E. N. Harvey—representing Princeton University
Professor M. H. Jacobs—representing The University of Pennsylvania
Professor Franz Schrader—representing Columbia University

Dr. Blakeslee was elected Chairman of the Committee and (by invitation) Dr. Charles Packard, Director of the Laboratory, Secretary.

Dr. Packard presented the Decennial Review containing a brief statement of the activities of the Laboratory for the years 1934–1943, and called attention to the nine exhibits.

The Committee examined the exhibits, and after full discussion, unanimously

VOTED That the Marine Biological Laboratory is performing valuable services in biological research.

It was the opinion of the Committee that it could perform a useful service to the Laboratory by making suggestions regarding its future development.

VOTED That the Committee understands and appreciates the high quality of the Board of Trustees of the Laboratory, but thinks it desirable that each class of Trustees should contain at least one biologist not closely associated with the work of the Laboratory.

Moved and seconded that a recommendation be formulated that some means be considered for effecting more frequent changes in the Board of Trustees.

The motion was lost.

VOTED That the Chairman appoint a sub-committee of three to report to the full Committee on the specific needs of the Laboratory.

The Chairman appointed Drs. Harvey, Dawson, and Packard.

VOTED That the Chairman appoint a sub-committee of three to draft a statement in support of the first motion, this to follow in general the form of the report of the 1934 Committee of Review.

The Chairman appointed Drs. Schrader, Jacobs, and Baitsell.

Afternoon Session.

VOTED To accept and adopt the following statement in support of the first motion.

The Marine Biological Laboratory is performing valuable services in biological research. Its record is especially commendable in view of the difficult conditions experienced during the past ten years. Despite the steady decrease in income from endowments, and the more recent handicaps involved in war conditions, the scientific activities of the Marine Biological Laboratory have been maintained at a high level.

With marked decrease in attendance due to wartime conditions, the standards of the courses of instruction have been maintained.

The Library, already recognized as one of the foremost in its field, has on a reduced budget been steadily improved.

Important research continues to be done. To compensate for a decrease in attendance there has been some utilization of the Laboratory facilities for war work.

As in the past, one of the important features of the Marine Biological Laboratory has been the close association of investigators working in different fields. Likewise, cooperation and association with the Woods Hole Oceanographic Institution, as well as with the local station of the U. S. Fish and Wild Life Service, has increased to a laudable extent.

VOTED To accept and adopt, as amended, the following statement of the subcommittee on Laboratory needs:

1. The committee notes that the income of the Marine Biological Laboratory has decreased while the needs have continually mounted. The budget has been balanced at the expense of upkeep and necessary improvements. Obviously the setting up of a sufficient reserve for future developments has been impossible. Additional income is urgently needed for the following specific purposes:
    a. Replacement of apparatus, boats, and other equipment now becoming obsolete.
    b. Repair and renovation of buildings.
    c. Payment of subscriptions to foreign journals now held in Europe.
    d. Probable adjustment of salaries to meet increased cost of living.
    e. Additional pensions.
    f. A naturalist to replace Mr. G. M. Gray, now retired.
    g. A fireproof building to replace the present wooden Laboratory buildings.
2. The Committee recognizes that the acquisition of funds for the above purposes and for additional endowment constitutes the most important problem confronting the Trustees of the Laboratory. In view of the anticipated increase in research activity after the war, these needs appear to be immediate and imperative.

The Committee directed the Secretary to inform the Trustees of the Laboratory of the above matters. The condensed report will be forwarded to the Bank as Trustee of the Endowment Funds; the full minutes will be published in the 1944 Annual Report of the Director.

The Committee adjourned at 4:45 P.M.

CHARLES PACKARD,
Secretary

August 9, 1944

3. TO THE COMMITTEE OF REVIEW

Gentlemen:

The first decennial review (1923–1933) included the period of rapid growth of the Laboratory. The Endowment Fund was set up; the chief building erected, more than doubling the space available for research; a special endowment for the

Library permitted a notable addition to its holdings of journals and books; a large amount of apparatus and other tools of research became available. As a result, the scientific activity of the Laboratory increased greatly. Toward the end of the period, the economic depression brought about a temporary slowing down of growth.

In the period now under review (1934–1943) growth was resumed. The Library overflowed the space allotted to it and spread into the new wing, a gift of the Rockefeller Foundation. The number of investigators increased, exceeding all previous records. The war has temporarily ended this growth. The Library now receives few foreign journals; the younger investigators are in active service or in war research; the classes, which have been maintained without interruption, are almost devoid of men. But the Laboratory has continued to offer all of its usual facilities to investigators and students. The current year (1944) shows a marked upward trend in attendance and scientific activity. The stability of the Laboratory during these periods of war and economic depression is noteworthy.

Personnel

Many important changes in personnel have occurred in the past ten years. Dr. F. R. Lillie retired as President of the Corporation, and was elected President Emeritus. In his stead, Mr. Lawrason Riggs, the Treasurer since 1924, was chosen President; and the office of Vice President, created in 1942, was filled by Dr. E. N. Harvey.

The following changes have occurred in the Board of Trustees:

(a) Died in Office: C. R. Stockard, D. H. Tennent.

(b) Elected Trustees Emeritus (having reached the age of seventy years):

G. N. Calkins, d. 1943
E. G. Conklin
B. M. Duggar
W. E. Garrey
Caswell Grave, d. 1944
M. J. Greenman, d. 1938
R. G. Harrison
H. S. Jennings
C. E. McClung
S. O. Mast
A. P. Mathews
W. J. V. Osterhout
G. H. Parker
W. M. Wheeler, d. 1937

(c) Elected Trustees:

E. G. Ball, Assoc. Prof. Biol. Chem., Harvard Medical School
S. C. Brooks, Prof. of Zoology, University of California
D. E. S. Brown, Prof. of Physiology, N. Y. University Dental School
G. H. A. Clowes, Director of Research, Eli Lilly Laboratory
E. F. DuBois, Prof. of Physiology, Cornell Medical College
P. S. Galtsoff, Senior Biologist, U. S. Fish and Wild Life Service
Laurence Irving, Prof. of Biology, Swarthmore College
Columbus Iselin, Director, Woods Hole Oceanographic Institution
C. W. Metz, Prof. of Zoology, University of Pennsylvania
J. H. Northrup, Member, Rockefeller Institute

H. H. Plough, Prof. of Biology, Amherst College
Franz Schrader, Prof. of Zoology, Columbia University
E. W. Sinnott, Prof. of Botany, Yale University
A. H. Sturtevant, Prof. of Genetics, Calif. Institute Technology
W. R. Taylor, Prof. of Botany, University of Michigan
B. H. Willier, Prof. of Zoology, Johns Hopkins University

Dr. M. H. Jacobs, appointed Director in 1926, resigned in 1937. Dr. Charles Packard was made Assistant Director in that year, and Director in 1939. Since 1942 he has been Resident Director.

Our investigators and students are drawn from institutions widely distributed throughout the country (cf. map, Exhibit 3). In addition to universities and colleges, 36 Medical Schools and Hospitals have sent representatives; 9 Research Institutes, a number of Federal and State services, and industrial laboratories are also represented. A complete list of all of these various institutions is found in Exhibits 4 and 5.

Statistics of attendance for the period under review are shown in Exhibit 3. The chart indicates the annual attendance since 1888 when the Laboratory was founded. The term "New Investigators" refers to those who work here for the first time; "Returning Investigators" are those who have previously spent one or more seasons at the Laboratory. The effect of the first world war and of the present war; of periods of economic depression; and of expansion in research facilities, can be seen.

An incomplete list of publications from this Laboratory is found in Exhibit 8. The scientific record of students attending the courses for the years 1918–1931 is also a part of this Exhibit since it indicates their continuing interest and success in biological research and teaching.

The Laboratory is in full operation and is open for your inspection.

Respectfully submitted,

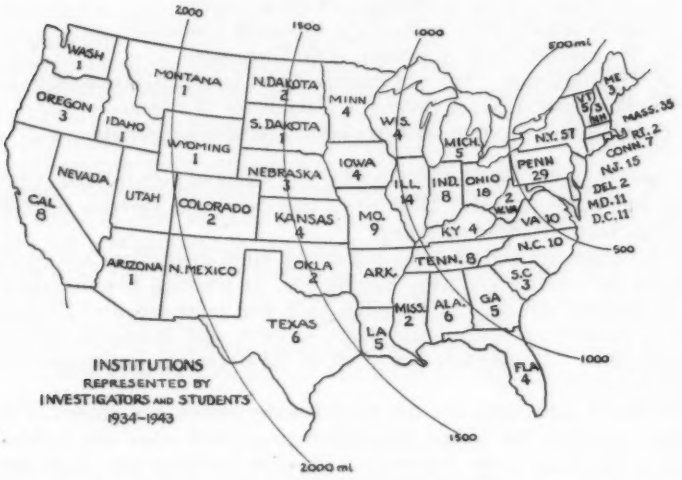
LAWRASON RIGGS, *President*
CHARLES PACKARD, *Director*

EXHIBITS

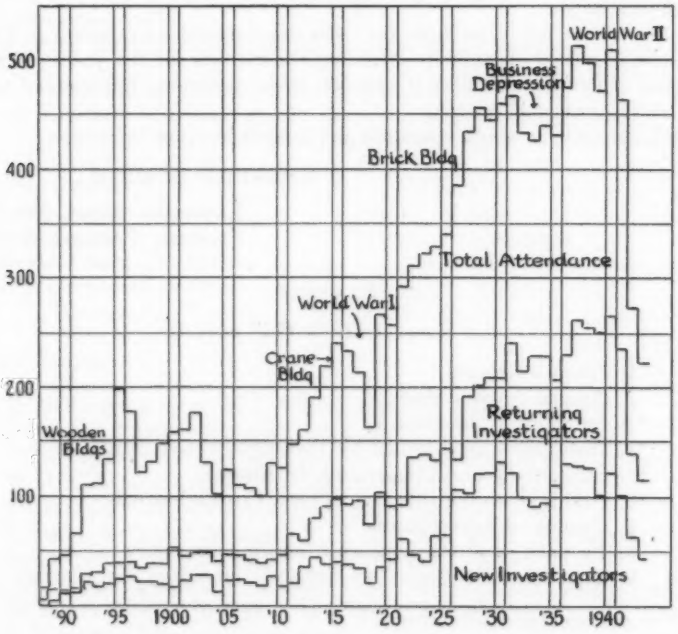
For the years 1934–1943 inclusive

1. Annual Reports
2. Annual Announcements
*3. Statistics of Attendance
*4. Institutions represented by Investigators and Students
*5. Subscribing and Cooperating Institutions
*6. Additions to the Library. Check List of Journals
7. Catalog of Investigators
8. Partial List of Publications from the Laboratory
9. The Scientific Record of Students attending the Courses

* These exhibits appear in this Report.



Geographical distribution of institutions represented at the Marine Biological Laboratory 1934–1943



Attendance at the Marine Biological Laboratory 1888–1943

EXHIBIT 3

A Tabular View of Attendance 1934–1943

	1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Investigators—Total	323	315	359	391	380	352	386	337	201	160
Independent	222	208	226	256	246	213	253	197	132	89
Beginning	49	56	76	74	53	60	62	59	16	19
Research Ass'ts	52	51	57	61	81	79	71	50	25	17
Library Readers								31	28	35
Students—Total	131	130	138	133	132	133	128	131	74	68
Botany	13	6	10	9	12	9	10	15	8	—
Embryology	30	33	34	35	34	36	34	37	24	13
Physiology	23	20	22	16	22	21	22	24	6	8
Protozoology	11	16	17	16	10	12	7	—	—	—
Zoology	54	55	55	57	54	55	55	55	36	47
Total Attendance less double registrations	439	429	473	511	496	471	507	461	273	222
Institutions represented	131	143	158	165	151	162	148	144	126	116
By investigators	98	111	120	134	125	132	112	102	83	71
By students	75	70	77	79	67	72	79	72	43	41
Schools and Academies										
By investigators	1	—	2	3	4	2	1	5	2	1
By students	5	3	3	2	1	2	2	2	—	1
Foreign Institutions										
By investigators	4	7	9	16	14	8	2	3	—	2
By students	1	1	5	—	3	1	1	1	—	—

EXHIBIT 4

Institutions Represented by Investigators and Students 1934–1943

A. Universities and Colleges

The figures after the name of the institution refer to the year the institution was first represented at the Laboratory

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Adelphi College	N. Y. '38					1					
Agnes Scott College	Ala. '15		1	1		1	1			1	
Akron, University of	Ohio '15	1									
Alabama, University of	Ala. '20		1	1	2			1	2		
Alabama University Medical	'37				1	1					
Alabama Polytech. Inst.	Ala. '38					1					
Albany Medical Coll.	N. Y. '31						1	1			
Albion College	Mich. '92		2	1	1		1				
American Internat. Coll.	Mass. '42									1	
American University	D. C. '31	3	2	2	1	1			1		
Amherst College	Mass. '13	2	5	11	10	6	8	6	6	7	1
Antioch College	Ohio '23				1						
Arizona, University of	Ariz. '25							1			
Assumption College	Mass. '42									1	
Atlanta University	Ga. '34	1	1	3	1		1			1	
Baldwin-Wallace Coll.	Ohio '35		1								
Bard College	N. Y. '35		1	1	2	1					
Barnard College	N. Y. '96	6	3	4	4	3	1	3	2		1
Baylor Univ. Medical	Tex. '42									1	
Beloit College	Wis. '96							1			
Bennington College	Vt. '35		2					1		1	1
Berea College	Ky. '28		1								
Birmingham-South. Univ.	Ala. '26						1				
Boston College	Mass. '38					1					
Boston University	Mass. '17						1				
Boston University Medical	Mass. '37				1						
Boston Teachers Coll.	Mass. '37				1						
Bowdoin College	Me. '93	2	1	1	1	1	1	1	1	1	
Bradley Poly. Inst.	Ill. '96	1									
Bridgewater State T. C.	Mass. '38					2					
Brooklyn College	N. Y. '32	7	5	9	7	2	4	7	3	1	1
Brown University	R. I. '90	1		1	3	3	7	7	7	1	

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Bryn Mawr College	Penn. '88	1	1		1		5	2	4		
Buffalo, University of	N. Y. '95						2				
Buffalo University Medical	N. Y. '39						1				
Butler University	Ind. '16		2	1							
California Inst. Tech.	Cal. '29	2	3	2	6	3	3	2	4	2	2
California, Univ. of	Cal. '00		1	3		2	3	5	4	5	1
Canisius College	N. Y. '37				1	1	2	4	2	4	
Carnegie Inst. Tech.	Penn. '09	1			1						
Catholic Univ. of Amer.	D. C. '42									2	2
Central State T. C.	Okla. '36			1							
Centre College	Ky. '37				1						
Charleston, Coll. of	S. C. '06			1			1	1			1
Chestnut Hill College	Penn. '41								2	2	
Chicago, University of	Ill. '92	10	7	6	7	9	5	13	8	2	3
Chicago University Medical	Ill. '40							1	1		
Cincinnati, Univ. of	Ohio '92	7	9	7	8	7	5	3	7	1	1
Cincinnati Univ. Medical	Ohio '35		3	2	3	3	5	3	1		
Clark University	Mass. '88	1	2	1	1	1	2				
Coe College	Iowa '20									1	
Colby College	Me. '99			1							
Colgate University	N. Y. '98					1					
College of Scholastica	Minn. '39						1				
College City of N. Y.	N. Y. '94	7	2	6	8	5	3	5	5		
Colorado, Univ. of	Col. '14	2	1								
Colorado Univ. Medical	Col. '25							1			
Columbia University	N. Y. '91	28	25	22	20	21	18	18	11	8	5
Columbia University Medical	N. Y. '94	5	5	5	7	8	5	3	2	5	4
Connecticut College	Conn. '20			2	2	2	3	3	2	2	1
Converse College	S. C. '38					1					
Cornell University	N. Y. '91	1		2	2	3	3	3	5	4	1
Cornell University Medical	N. Y. '09	12	12	9	11	8	10	6	5	1	1
Dartmouth College	N. H. '96	4	3	4	4	4	4	4	3	2	1
Davis and Elkins Coll.	W. Va. '41								1		
Delaware, Univ. of	Del. '98							1			
Delta State T. C.	La. '36			1							
DePaul University	Ill. '41								1		

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
DePauw University	Ind. '89	7	6	7	3	4	2	5	3	1	3
Dillard University	La. '42									1	1
Drew University	N. J. '38					1	2	1		2	
Drury College	Mo. '43										1
Duchesne College	Neb. '35		1								
Duke University	N. C. '25	5	2	1	8	7		3	2	1	1
Earlham College	Ind. '01				1		1				
East Carolina T. C.	N. C. '38					1					
Edgewood Park Jr. Coll.	N. C. '34	1									
Elizabethtown Coll.	'38					1					
Elmira College	N. Y. '21					1	2				
Elon College	N. C. '40							1			
Emory University	Ga. '97			1			2				
Emory Junior Coll.	Ga. '33				1						
Fisk University	Tenn. '31							1			
Flat River Jr. Coll.	'28			1							
Flora Macdonald Coll.	N. C. '25				1						
Florida, University of	Fla. '41								1		
Florida State College	Fla. '39						1				
Florida Coll. for Women	Fla. '29						1				
Fordham University	N. Y. '19								3	3	8
Franklin Marshall Coll.	Penn. '91			1			1	1	1		
George Washington Univ. Medical	D. C. '14	1	1	1	1	1	2	2	1		2
Georgia, University of, Medical	Ga. '37				1						
Gettysburg College	Penn. '39						1	1	2		
Goucher College	Md. '94	3	2	4	3	4	5	3	3	2	3
Great Falls Norm. Coll.	Mont. '38					1	1	1			
Grinnell College	Iowa '95	1									
Gulf Park College	Ky. '36			1							
Hahneman Medical Coll.	N. Y. '38					1	1	2			
Hamilton College	N. Y. '91		2	2	2		1		1		
Harvard University	Mass. '89	10	14	11	15	11	16	12	14	4	1
Harvard University Medical	Mass. '89	1	2	3	2	4	2	6	3	3	2
Haverford College	Penn. '89								1		
Heidelberg College	Ohio '91									1	
Herzl Junior College	Ill. '43										1

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Hood College	Md. '15	1									
Howard University	D. C. '09	1	1	1	2		1				
Hunter College	N. Y. '14	7	3	4	2	1	4	1		3	
Illinois, Univ. of	Ill. '90	5	6	2	4	2	1	5	4	3	2
Indiana University	Ind. '89			1				2	2		
Iowa, State Univ. of	Iowa '94	6	3	6	3	3	5	5	6	2	1
Iowa, State College of	Iowa '19	2	1	3	3	3	5	1	1		
Johns Hopkins Univ.	Md. '89	9	3	8	11	17	12	10	11	8	2
Johns Hopkins Univ. Medical	Md.	2	2	4	2	4	5	1			
Kansas, University of	Kan. '90	1		2	1			1			
Kansas State College	Kan. '26									1	
Kansas State T. C.	Kan. '34	1									
Kent State University	Ohio '30							1			
Kenyon College	Ohio '96				1	1					
Lander College	S. C. '38					1					
LaVerne College	Cal. '34	1									
Leland Stanford Univ.	Cal. '91			1		1	3	7	1	1	
Lincoln University	Penn. '01		1								
Long Island Univ.	N. Y. '29	3	3	3	2	1	1	1		1	
Long Island Univ. Medical	N. Y. '19									1	
Loyola Univ. Medical	La. '31	1		1					1		
Maine, University of	Me. '95	1	1	1	1						
Marquette University	Wis. '36			1							
Maryland, Univ. of	Md. '41								1		
Maryland Univ. Medical	Md. '96			8	11	13	11	4	3	5	1
Mass. State College	Mass. '89				1	1	1	1	2	2	1
Mass. Inst. Technology	Mass. '88	1					1	1	2	2	1
Miami University	Ohio '91				1	3	3	3	3	2	
Michigan, Univ. of	Mich. '88	4	3	2	2	4	3	6	9	2	2
Michigan Agric. Coll.	Mich. '10					1					
Middlebury College	Vt. '95		1			1	1				
Middlesex College	Mass. '37				1						
Minnesota, Univ. of	Minn. '98		1	2	7	3	2	3	2	1	
Mississippi, Univ. of	Miss. '99							1			
Missouri, Univ. of	Mo. '95	3	7	10	1	7	1	5	4	1	
Missouri State T. C.	Mo. '43										1

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Moberly Junior Coll.	Wis. '40							1			
Morehouse College	Ga. '27						1				
Morristown College	Tenn. '37				1						
Mt. Holyoke College	Mass. '88	3	3	6	7	8	7	4	8	8	3
Mt. Mercy College	Penn. '39						1				
Mt. St. Louis Coll.	'34	1									
Mundelein University	Ill. '39						1		1		
National Park Coll.	Md. '42									1	
Nebraska, Univ. of Medic.	Neb. '34	1									
New Hampshire State U.	N. H. '00					1					
New Jersey College for Women	N. J. '28	4	3	2	1	3	1		1		1
New Jersey State T. C.	N. J. '31	2	4	6	5	3	4	3	1		
New Rochelle, Coll. of	N. Y. '34	1				1	1				1
New York University	N. Y. '96	4	3	10	7	7	2	5	7	2	2
New York University Medical	N. Y. '25	6	2	6	10	7	3	9	5	7	6
New York University Wash. Sq.	N. Y. '24	10	10	10	10	11	10	12	8	9	5
Newark, University of	N. J. '41								1		
Newark State T. C.	N. J. '41								1		
North Carolina, Univ. of	N. C. '99		1	1	1	2	3	1	3		3
North Carolina Coll. for Negroes	N. C. '40							1			
N. C. State College	N. C. '31		1	2							
N. C. Womens College	N. C. '22			1	1						
N. Dakota State Univ.	N. D. '93	2	1	1							
N. Dakota Agric. Coll.	N. D. '23		1					1			
N. Texas Agric. Coll.	Tex. '38					1					
Northwestern Univ.	Ill. '93	3	4	3	6	1	5	9	2		
Notre Dame Univ.	Ind. '21			1	1	1	1				
Oberlin College	Ohio '90	5	7	8	7	5	4	6	6	4	5
Ohio State Univ.	Ohio '90			1	4	10	3	7	8	5	2
Ohio University	Ohio '14				2						
Ohio Wesleyan Univ.	Ohio '91				1	1	2		2		1
Oklahoma, Univ. of	Okla. '09						1	1			
Oklahoma City, Univ. of	Okla. '37				1	1	1	1	1		
Pennsylvania, Univ. of	Penn. '91	37	38	35	31	28	26	32	27	17	17
Pennsylvania, Univ. of Medical	Penn.	6	3	5	6	5	8	8	6	6	3
Penn. Coll. for Women	Penn. '01	3	3	1	1	2	2	2		2	1

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Penn. State Coll.	Penn. '07						1				
Pittsburgh, Univ. of	Penn. '21	3	5	7	7	4	5	7	6	2	
Placer Junior College	Cal. '40							1			
Pomona College	Cal. '24			1							
Princeton University	N. J. '90	12	7	12	13	12	13	9	9	3	5
Providence College	R. I. '35		1	2							
Purdue University	Ind. '28		3	5	4	5	1	3	3		
Queens College	N. Y. '28					1	1	4	3		
Radcliffe College	Mass. '95	4	3	3	6	2	1	2	5	2	1
Randolph-Macon Coll.	Va. '89								1		
Reed College	Ore. '39						1				
Rensselaer Poly. Inst.	N. Y. '36			1	1				1		
Rice Institute	Tex. '16				1						
Richmond, College of	Va. '13				1	1			1	1	1
Rochester, Univ. of	N. Y. '92	8	10	4	7	5	5	6	6	2	4
Rochester, Univ. of Medical	N. Y. '35		5		2	2	1				
Russell Sage College	N. Y. '20				1		2	2	1	5	4
Rutgers University	N. J. '14	3		2	1	1	2	1	2	2	
St. Francis Xavier Coll.	Ill. '14	1		2							
St. Johns College	Md. '34	2	2	1	2	2	1				
St. Louis University	Mo. '03	1	1	2	3						
St. Louis University, Maryville	Mo. '37				1						
St. Thomas College	Minn. '35		1								
St. Vincent College	Penn. '24								1		
Sarah Lawrence College	N. Y. '32		4	5	1				2	1	
Seton Hall College	N. J. '35		1	2							
Seton Hill College	Penn. '29				1	1		1	3	1	4
Simmons College	Mass. '07	1	1	1	1	1		1			
Skidmore College	N. Y. '22	1	2	2	2				1	1	
Smith College	Mass. '92	1	7	5	2	2	2	2	5	4	3
J. C. Smith University	N. C. '34	1	1	1	1	2					
Southern California, University of	Cal. '96						2				
Southern Oregon State Normal	Ore. '38					1					
Southwestern Univ.	Tenn. '22		1					1			
Spring Hill College	Ala. '38					1					
Springfield College	Mass. '40							2	1		
Stephens College	Mo. '36			1							

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Swarthmore College	Penn. '88	6	4	7	6	11	9	7	4		1
Sweet Briar College	Va. '09		2							1	
Syracuse University	N. Y. '94		1	1	2			1	1	1	
Syracuse University Medical	N. Y. '99	2	2	1	1	1	2	4	2	1	
Temple University	Penn. '97	2	2	1	4	3	2	3	4	2	3
Tennessee, Univ. of	Tenn. '95	3									
Tennessee, Univ. of Medical	Tenn. '35		2	2	2						
Texas, University of	Tex. '95					1		1	1		
Texas, University of Medical	Tex. '36			1	1	1					
Texas Christian Univ.	Tex. '21									1	
Toledo, Univ. of	Ohio '17					2	2		1		
Tougaloo College	Miss. '39						1				
Trinity College	Conn. '00	1	2	2	2	2	2	1	1	1	
Tufts College	Mass. '92			1			2	1	2		2
Tulane University	La. '16								1		
Tulane Newcomb Coll.	La. '13	1							1		
Union College	N. Y. '15	2	1	2	1	3	2	4	3	2	1
Union College	Ky. '39						1				
Ursinus College	Penn. '95		1								
Vanderbilt University	Tenn. '91		1					1			
Vanderbilt University Medical	Tenn. '34	4	3	3	3	4	3	2	1	2	1
Vassar College	N. Y. '88	4	2	3	2	6	3	5	7	6	6
Vermont, University of	Vt. '15		1					1			
Vermont, University of Medical	Vt. '38					2	1	1	1	1	1
Vermont State Normal	Vt. '40							1	1	1	1
Villanova College	Penn. '39						1	6	5	1	
Virginia, Univ. of	Va. '91		2	1	1	2	2				
Virginia, Univ. of Medical	Va.	2	2	2	4	2	2	2	1		
Virginia State T. C.	Va. '34	1									
Virginia Intermont Coll.	Va. '41								1		
Wabash College	Ind. '07	1	2	3	1	1		2	2		
Washburn College	Kan. 40'							1			
Washington University	Mo. '00	1	2	8	12	9	10	8	5	5	9
Washington University Medical	Mo.		2		6	3	2	3	1		
Washington, Univ. of	Wash. '15					1					
Washington and Jefferson College	Penn. '11	1	1	1	1	2	1	2	1	2	1

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Washington and Lee	Va. '17			1							
Wayne University	Mich. '34	1	1								
Wellesley College	Mass. '88	3	5	3	3	4	2	2	2	5	1
Wesleyan University	Conn. '89	6	5	3	5	6	4	7	10	3	1
West Virginia Univ.	W.Va.'01	1					1		1		
Westbrook Junior Coll.	Conn. '40							1			
Western College	Ohio '98					1					
Western Reserve Univ.	Ohio '95	3	1			4	1		1		
Wheaton College	Mass. '18	1	2	1	1	4	3	4	2	2	3
Whitman College	Ore. '43										2
William and Mary	Va. '22			1	1	1	1	1	1		
Williams College	Mass. '92	1	4	2	1	5	4	3	5	1	
Wilson College	Penn. '24	1	1	2	2	2	1	1	1	1	1
Wisconsin, Univ. of	Wis. '98	4	4	2	5		1	2	2		2
Womens Medical College	Penn. '92	1		3	3		2	3			
Wooster College	Ohio '13				1						
Wright Junior College	Ill. '41								1		
Wyoming, Univ. of	Wyo. '29								1		
Yale University	Conn. '91	14	8	6	16	10	9	11	15	5	1
Yale University Medical	Conn. '38					1		4	2	1	

B. HIGH SCHOOLS, ACADEMIES, ETC.

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Abraham Lincoln H. S.	N. Y.			1							
Agnes Irwin School	N. Y.			1							
Annapolis H. S.	Md.	1									
Berkshire School	Mass.								1		
Birmingham H. S.	Ala.	1									
Boston H. S.	Mass.	1							1		
Bronxville H. S.	N. Y.		1								
Caldwell H. S.	N. J.			1							
Central H. S.	D. C.				1						
Chicago H. S.	Ill.						1				
Choate School	Conn.							1	1		
Dana Hall	Mass.			1							
Darrow School	N. Y.						1				
Deerfield Academy	Mass.						1				

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Eastern District H. S.	N. Y.				1						
Emma Willard School	N. Y.										1
Exeter Academy	N. H.							1	1	1	1
Galesburg H. S.	Ill.	1									
Grand Falls H. S.	Can.				1						
Groton School	Conn.			1						1	
Hallahan H. S.	Penn.							1			
Hawthorne H. S.	N. J.			1							
Hyde School	Mass.			1							
Hyde Park School	Ill.				1			1	1		
Jenkintown H. S.	Penn.		1								
Knox School	N. Y.			1							
Lawrenceville School	Mass.								1		
Los Angeles H. S.	Cal.								1		
Milton Academy	Mass.	1		1		4	4	5	4		
Nativity H. S.	Mass.							1			
Negaunee H. S.	Mich.	1									
Oradell H. S.	N. J.					1					
Pennsgrove School	Penn.								1		
Potomac School	D. C.								1		
St. Andrews School	Del.						1				
St. Catherine School	Va.							1			
St. Joseph's Seminary	N. Y.									1	
St. Mary of the Lake Sem.	Ill.								1		
Scott, H. S.	Ohio	1									
Society of the Divine Word	Mass.									1	
Straubenmiller H. S.	N. Y.				1						
Theo. Roosevelt H. S.	N. Y.			1	1						
Union H. S.	N. J.	1									
Union City H. S.	Tenn.	1									
University H. S.	Minn.		1								
Vineland Training School	N. J.		1	1							
Walton H. S.	N. J.				1						
Washington H. S.	D. C.						1				
Weequahic H. S.	N. J.			1	1	1				1	
Westtown Friends School	Penn.							1			

EXHIBIT 4—*Continued*

C. INSTITUTES, FOUNDATIONS, ETC.

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
American Mus. Nat. Hist.	N. Y. '09		1								
Arlington Chemical Co	'31	1	1								
Barnard Skin and Cancer Hospital	Mo.								1		
Beth Israel Hospital	N. Y. '42										
Biol. Institute, Phil.	Penn. '39						1				
Carnegie Institute											
Cold Spring	N. Y. '14		1								
Washington	D. C. '15	2				4	2	1	1		
Frick Education Comm.	'42									1	
Guggenheim Foundation	'40							1			
Guggenheim Dental Clinic	'43										1
Journ. Industrial and Engineering Chemistry	'28	1	1	1	1	1	1	1	1		
Internat. Cancer Research Foundation	'37				1						
Eli Lilly Company	Ind. '19	5	4	4	4	5	5	4	5	5	2
Marine Studios, Inc.	Fla. '42									1	
Memorial Hospital	N. Y. '26	2	1	1	2	3	3	2	1	1	1
Mt. Sinai Hospital	N. Y. '40							2	1	1	1
Nat. Cancer Institute	Md. '39						2				
Nat. Research Council	D. C.				1					1	
N. Y. State Agricult. Station	N. Y. '18				1						
N. Y. State Dept. Health	N. Y. '19	1	2	1	1	1	1	2	2	1	
Overly Biochemical Research Found.	N. Y. '43										1
Phila. Acad. Nat. Sci.	Penn. '89					1		1			
Rockefeller Institute	N. Y. '11	10	12	11	11	13	8	14	9	6	5
Rockefeller Foundation Fellowship		3		1		2	1				
Russell Sage Institute of Pathology	N. Y. '34	1					1	1			
St. Luke's Hospital	N. Y. '40							1			
U. S. Dept. Agriculture	D. C. '99					1					
U. S. Dept. Public Health	D. C. '20					1					
U. S. Fish and Wild Life Service	D. C. '42									2	2
Wistar Institute	Penn. '08	1	1	1	1		1				
Woods Hole Oceanographic Inst.	Mass. '43										

EXHIBIT 4—*Continued*

D. INSTITUTIONS OUTSIDE THE UNITED STATES

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Austria	Univ. of Innsbruck	1									
	Univ. of Vienna					2					
Belgium	Univ. of Ghent					1					
	Univ. of Liege					1					
	Belgian-American Educ. Foundation						3				
British Isles	Queens Coll. Belfast			1							
	Cambridge University	1			1	1	1				
	Trinity Coll. Dublin				1						
	Univ. of Edinburgh					1					
	Univ. of Leeds				1						
	Univ. of London				2			1	1	1	1
	Univ. Coll. London			1		1					
	Univ. Coll. Nottingham							1			
	Oxford University			1		1					
	British Fish. Service					1					
Canada	Acadia University N. S.	1									
	Univ. British Columbia			1							
	Dalhousie University, N. S.	2				1	1		1		
	McGill University, Ont.			1	2	3	2	3			1
	Univ. of Manitoba				1						
	Univ. of Montreal	1	1	1		1		1			
	Memorial University, N. F.		1								
	Coll. Ste. Marie, Montreal		1								
	Univ. of Toronto	6	7	6	3	5	4	5	4	4	1
	Univ. West. Ontario		1	1							
	Royal Soc. Canada			1							
Far East	Womens Medical College, Madras, India						1				
	Judson Coll. Rangoon, Burma						1				
China	Peking Union Med. Coll.						1				
Colombia	Cotton Res. Station										1
Denmark	Carlsberg Laboratory		1					1			
	Univ. of Copenhagen							1	1		
Egypt	Egyptian Educ. Commission		1								

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
France	Pasteur Institute, Paris					1					
	University of Paris				1						
	University of Strasbourg				1						
	University of Strasbourg Medical				1						
Germany	University of Berlin			1	1	1					
	Neurolog. Inst. Frankfurt				2						
	University of Munich			1							
Hungary	University of Debrescen				1						
	Budapest Univ. Medical		1								
	Franz Joseph University					1					
Italy	University of Padua		1		1						
Japan	Misaki Biolog. Inst.			1							
Norway	University of Oslo							1			
Peru	Guano Administration									1	
Poland	University of Lwow		1		1						
Russia	Moscow, Inst. Genetics			2							
Serbia	Belgrade Medical Coll.		1		1						
Spain	Barcelona Medical Coll.			1							
	University of Lund		1		1						
Sweden	Karolinska Inst. Stockholm		1		1						
	University of Stockholm	1		1							
Switzer-	Physiological Inst. Berne	1									
land	Zoological Inst. Berne				1						
	University of Geneva			1							
Uruguay	Ministry of Pub. Health										1
Cuba	University of Havana					2	2				

Summary

	1923–33	1934–43
Universities and Colleges	246	269
High Schools and Academies	30	50
Institutes, Foundations, etc.	56	31
Foreign Institutions	101	59
	433	409

EXHIBIT 5

Subscribing and Cooperating Institutions

A cooperating institution is one that has subscribed for the two preceding years, or that announces its intention of subscribing regularly. A subscribing institution is one that pays for one or more tables or rooms.

	1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
American University	x		x	x	x			x		
Amherst College	x	x	x	x	x	x	x	x	x	x
Atlanta University	x	x	x	x		x			x	x
Barnard College	x	x			x		x	x		
Belgian-Amer. Educ. Found.					x	x				
Bell Telephone Laboratory										x
Berea College	x	x								
Beth Israel Hospital									x	
Biological Institute, Phila.							x	x	x	x
Bowdoin College	x	x	x	x	x	x	x	x	x	x
Brooklyn College							x	x	x	x
Brown University				x	x	x	x	x	x	
Bryn Mawr College	x	x	x	x	x	x	x	x	x	
Buffalo University Medical						x				
Butler University		x								
C. R. B. Educational Found.				x						
California Inst. Technol.		x	x	x	x	x	x	x		
Canisius College						x	x	x	x	
Carnegie Inst. Washington					x	x				
Catholic Univ. of America										x
Chicago, University of	x	x	x	x	x	x	x	x	x	x
Chicago, University of Medical		x								
Children's Hospital Cincinnati		x								
Chinese Educational Mission	x									
Christ Hospital, Cincinnati		x								
Cincinnati, University of	x	x	x	x	x	x	x	x	x	x
Columbia University	x	x	x	x	x	x	x	x	x	x
Columbia University Medical		x		x	x	x	x	x	x	x
Commonwealth Fund	x									x
Connecticut College					x	x				
Cornell University	x	x	x	x			x	x	x	x
Cornell University Medical	x	x	x	x	x	x	x	x	x	x
Dalhousie University	x				x	x				
Dartmouth College				x	x	x				
De Pauw University	x	x	x	x	x	x	x	x		
Drew University					x	x			x	

EXHIBIT 5—*Continued*

	1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Duke University	x	x	x	x	x	x	x	x	x	x
Eli Lilly Research Lab.	x	x	x	x	x	x	x	x	x	x
Elmira College					x	x				
Fisk University							x			
Fordham University								x	x	x
Frick Educational Comm.									x	
General Education Board	x	x	x	x	x					
Georgia, Univ. of Medical				x						
Goucher College	x	x	x	x	x	x	x	x	x	x
Hamilton College		x	x	x		x		x		
Harvard University	x	x	x	x	x	x	x	x	x	x
Harvard University Medical	x	x	x	x	x	x	x	x	x	x
Heidelberg College									x	
Howard University						x				
Hunter College	x	x	x	x	x	x	x		x	
Illinois, University of	x	x	x	x	x	x	x	x	x	x
Indiana University								x		
Industrial and Engin. Chem.	x	x	x	x	x	x	x	x	x	x
Iowa, State Univ. of	x	x	x	x	x	x	x	x	x	x
Iowa, State College of	x	x	x	x	x	x				
Johns Hopkins University	x	x	x	x	x	x	x	x	x	x
Johns Hopkins University Medical	x					x	x			
Johnson Foundation			x							x
Josiah Macy Foundation								x		
Julius Rosenwald Fund									x	
Kansas, University of	x		x				x			
Kenyon College				x	x					
Leland Stanford Univ.							x			
Lincoln University		x								
Long Island University	x	x	x	x	x	x	x		x	
Loyala Univ. Medical								x		
McGill University						x				
Markle Foundation									x	
Maryland, Univ. of Medical			x	x	x	x		x	x	x
Marine Studios, Inc.									x	
Mass. General Hospital										x
Mass. State College				x	x	x	x	x	x	
Memorial Hospital, N. Y.		x	x	x	x	x				
Michigan, University of								x		
Minnesota, University of			x	x	x					
Missouri, University of		x	x		x	x	x	x	x	
Morehouse College	x	x	x	x		x			x	

EXHIBIT 5—*Continued*

	1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Mt. Holyoke College	x	x	x	x	x	x	x	x	x	x
Mt. Sinai Hospital							x	x	x	
Mundelein University						x		x		
National Research Council									x	
N. Y. Dept. of Health	x	x	x	x	x	x	x	x	x	
New York University	x	x	x	x	x	x	x	x	x	x
New York University Medical	x	x	x	x	x		x	x	x	x
New York University Wash. Square	x	x	x	x	x	x	x	x	x	x
N. Carolina Coll. for Negroes							x			
Northwestern University	x	x	x	x	x	x	x	x		
Notre Dame University					x	x				
Oberlin College	x	x	x	x	x	x	x	x	x	x
Ohio State University							x	x	x	x
Ohio Wesleyan University								x		
Penn. College for Women	x	x	x	x	x	x	x		x	x
Pennsylvania, Univ. of	x	x	x	x	x	x	x	x	x	x
Pennsylvania, Univ. of Medical	x	x	x	x	x	x	x	x	x	x
Pittsburgh, Univ. of	x	x	x	x	x	x	x	x	x	
Princeton University	x	x	x	x	x	x	x	x	x	x
Purdue University		x	x	x	x	x	x	x		
Radcliffe College	x	x	x	x	x	x	x	x	x	x
Rensselaer Poly. Inst.			x							
Rice Institute				x						
Rochester, University of	x	x	x	x	x	x	x	x	x	x
Rochester, University of Medical	x	x		x	x					
Rockefeller Foundation	x	x	x	x	x	x	x			
Rockefeller Institute	x	x	x	x	x	x	x	x	x	x
Royal Egyptian Foundation		x								
Russell Sage College							x	x	x	
Rutgers University	x		x	x	x	x	x	x	x	x
St. Elizabeth, College of	x									
St. Francis Xavier College			x							
St. Johns College		x		x	x	x				
Sarah Lawrence College								x		
Seton Hall College		x	x							
Seton Hill College				x				x		x
Smith College	x	x	x	x	x	x	x	x	x	x
J. C. Smith University	x	x								
Spring Hill College					x					
Springfield College							x	x		
Swarthmore College	x	x	x	x	x					
Sweet Briar College									x	

EXHIBIT 5—*Continued*

	1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Syracuse University	x	x	x	x	x		x	x	x	
Syracuse University Medical						x	x			
Temple University	x	x	x	x	x	x				
Toledo, University of					x	x		x		
Tufts College	x	x	x		x	x	x	x	x	x
Tulane University								x		
Tulane University Newcomb Coll.	x							x		
Union College, N. Y.		x	x	x	x			x	x	
Union College, Ky.						x	x			
U. S. Fish and Wild Life Serv.										x
Vanderbilt University		x								
Vanderbilt University Medical	x	x	x	x	x	x	x	x	x	
Vassar College	x	x	x	x	x	x	x	x	x	x
Vermont, University of		x			x					
Villanova College						x	x	x	x	
Virginia, University of	x	x	x	x	x	x	x	x		
Wabash College	x	x	x	x	x		x	x		
Washington University				x	x	x	x	x	x	x
Washington University Medical				x	x	x	x	x		
Wellesley College	x	x	x	x	x	x	x	x	x	x
Wesleyan University	x	x	x	x	x	x	x	x	x	
Western Reserve University		x			x	x				
Wheaton College	x	x	x	x	x	x	x	x	x	x
William and Mary College					x	x				
Williams College					x	x				
Wilson College	x	x	x	x	x	x	x			
Wisconsin, University of		x	x	x						
Wistar Institute	x	x	x	x						
Woods Hole Oceanographic Inst.									x	x
Yale University	x	x	x	x	x	x	x	x	x	x
Yale University Medical							x	x		

EXHIBIT 6

Additions to the Library

	Bound volumes	Reprints	Sets completed	Partially completed	New journals	Classics	Budget
1934	1,138	5,028	8	16	18		$20.325
1935	1,622	4,478	24	17	55		22.444
1936	2,107	3,339	29	12	28		22.510
1937	1,155	7,042	24	20	21		22.029
1938	1,455	6,905	33	30	15		19.515
1939	1,239	3,850	33	24	21		22.149
1940	1,561	3,528	20	46	21		17.923
1941	1,482	3,321	24	91	25	4	16.964
1942	1,758	3,097	45	161	13	23	15.332
1943	1,008	7,927	10	51	10	5	11.047
Total added	14,525	48,515	250	468	227	32	
Total in Libr.	51,945	129,723	1800 approx.	600 approx.			

4. THE STAFF, 1944

Charles Packard, Director, Marine Biological Laboratory, Woods Hole, Massachusetts.

Senior Staff of Investigation

E. G. Conklin, Professor of Zoology, Emeritus, Princeton University.
Frank R. Lillie, Professor of Embryology, Emeritus, The University of Chicago.
Ralph S. Lillie, Professor of General Physiology, Emeritus, The University of Chicago.
C. E. McClung, Professor of Zoology, Emeritus, University of Pennsylvania.
S. O. Mast, Professor of Zoology, Emeritus, Johns Hopkins University.
A. P. Mathews, Professor of Biochemistry, Emeritus, University of Cincinnati.
T. H. Morgan, Director of the Biological Laboratory, California Institute of Technology.
G. H. Parker, Professor of Zoology, Emeritus, Harvard University.

ZOOLOGY

I. Consultants

T. H. Bissonnette, Professor of Biology, Trinity College.
L. L. Woodruff, Professor of Protozoology, Yale University.

II. Instructors

J. B. Buck, Assistant Professor of Zoology, University of Rochester, in charge of course.
T. H. Bullock, Instructor in Neuro Anatomy, Yale University.
W. D. Burbanck, Associate Professor of Biology, Drury College.
C. G. Goodchild, Associate Professor of Biology, Southwest Missouri State Teachers College.
John H. Lochhead, Instructor in Zoology, University of Vermont.
Madelene E. Pierce, Assistant Professor of Zoology, Vassar College.
W. M. Reid, Assistant Professor of Biology, Monmouth College.
Mary D. Rogick, Professor of Biology, College of New Rochelle.

III. Laboratory Assistants

Gene Lehman, Teaching Fellow Zoology, University of North Carolina.
Mary E. Banks, Washington University.

EMBRYOLOGY

I. Consultants

L. G. Barth, Assistant Professor of Zoology, Columbia University.
H. B. Goodrich, Professor of Biology, Wesleyan University.

II. Instructors

Viktor Hamburger, Professor of Zoology, Washington University, in charge of course.
Donald P. Costello, Assistant Professor of Zoology, University of North Carolina.
Ray L. Watterson, Assistant Professor of Zoology, University of California.

PHYSIOLOGY

I. Consultants

William R. Amberson, Professor of Physiology, University of Maryland, School of Medicine.
Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Merkel H. Jacobs, Professor of Physiology, University of Pennsylvania.

II. Instructors

Arthur K. Parpart, Associate Professor of Biology, Princeton University, in charge of course.
Robert Ballentine, Lecturer in Zoology, Columbia University.
Arthur C. Giese, Associate Professor of Biology, Stanford University (absent in 1943).
Rudolf T. Kempton, Professor of Zoology, Vassar College.

BOTANY

I. Consultants

S. C. Brooks, Professor of Zoology, University of California.
B. M. Duggar, Professor of Plant Physiology, University of Wisconsin.

II. Instructors

Wm. Randolph Taylor, Professor of Botany, University of Michigan, in charge of course.
Hannah Croasdale, Technical Assistant, Dartmouth College.

EXPERIMENTAL RADIOLOGY

G. Failla, Memorial Hospital, New York City.
L. Robinson Hyde, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian
Deborah Lawrence Mrs. Elon H. Jessup Mary A. Rohan

APPARATUS DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager
J. D. Graham

CHEMICAL DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager

SUPPLY DEPARTMENT

James McInnis, Manager

Ruth Crowell — Grace M. Kennerson
M. B. Gray — W. E. Kahler — G. Lehy
A. M. Hilton — A. W. Leathers — F. N. Whitman

GENERAL OFFICE

F. M. MacNaught, Business Manager
Polly L. Crowell — Mrs. Carlotta I. Cowin

GENERAL MAINTENANCE

T. E. Larkin, Superintendent
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R. W. Kahler — T. E. Tawell
A. J. Pierce

THE GEORGE M. GRAY MUSEUM

George M. Gray, Curator Emeritus

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5. INVESTIGATORS AND STUDENTS

Independent Investigators, 1944

Abell, Richard G., Assistant Professor of Anatomy, University of Pennsylvania.
Addison, William H. F., Professor of Normal Histology and Embryology, University of Pennsylvania.
Allee, W. C., Professor of Zoology, University of Chicago.
Anderson, Rubert S., Assistant Professor, University of Maryland.
Ballentine, Robert, Instructor, Columbia University.
Barth, L. G., Assistant Professor of Zoology, Columbia University.
Bartlett, James H., Associate Professor of Theoretical Physics, University of Illinois.
Belcher, Jane C., Instructor, Sweet Briar College.
Berger, Charles A., Director, Biological Laboratory, Fordham University.
Bergmann, Werner, Associate Professor, Yale University.
Bertholf, Lloyd Millard, Dean of Faculty and Professor of Biology, Western Maryland College.
Brown, Dugald S., Professor of Physiology, New York University.
Brownell, Katharine A., Research Associate, Ohio State University.
Buck, John B., Assistant Professor of Zoology, University of Rochester.
Budington, Robert A., Professor of Zoology, Emeritus, Oberlin College.
Bullock, Theodore H., Instructor in Neuro-Anatomy, Yale University.
Burbanck, W. D., Associate Professor of Biology and Chairman of Department, Drury College.

CHAMBERS, ROBERT, Research Professor of Biology, New York University.
CLARK, ELEANOR L., Voluntary Research Worker, University of Pennsylvania, Medical School.
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CLEMENT, A. C., Associate Professor of Biology, College of Charleston.
CLOWES, G. H. A., Director of Research, Lilly Research Laboratories, Eli Lilly and Company.
CONKLIN, EDWARD G., Professor Emeritus of Biology, Princeton University.
COPELAND, MANTON, Professor of Biology, Bowdoin College.
COSTELLO, DONALD P., Professor of Zoology, University of North Carolina.
CRAMPTON, H. E., American Museum of Natural History.
CROASDALE, HANNAH T., Technical Assistant in Zoology, Dartmouth College.
CUTKOMP, LAURENCE K., Research Fellow in Zoology, University of Pennsylvania.
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FAILLA, G., Professor of Radiology, College of Physicians and Surgeons, Columbia University.
FROEHLICH, ALFRED, Associate, May Institute for Medical Research, Cincinnati, Ohio.
GALTSOFF, PAUL S., Senior Biologist, U. S. Fish and Wildlife Service.
GARREY, W. E., Professor of Physiology, Vanderbilt University, School of Medicine.
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GLASER, OTTO C., Professor of Biology, Amherst College.
GOODCHILD, C. G., Associate Professor of Biology, State Teachers College.
GORBMAN, AUBREY, Instructor in Biology, Wayne University.
GRAND, C. G., Research Associate, New York University.
GRELL, SISTER MARY, Student and Investigator, Fordham University, New York.
HALLOCK, FRANCES A., Associate Professor, Hunter College.
HAMBURGER, VIKTOR, Professor of Zoology, Washington University.
HARNLY, MORRIS H., Associate Professor, Washington Square College, New York University.
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HARVEY, E. NEWTON, Professor of Physiology, Princeton University.
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LEWIS, WARREN H., Member, The Wistar Institute of Anatomy and Biology.
LIEBMAN, EMIL, Research Fellow, Princeton University.
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MACLEAN, BERNICE L., Assistant Professor, Department of Biological Sciences, Hunter College.
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PIERCE, MADELENE E., Assistant Professor of Zoology, Vassar College.
POLLISTER, ARTHUR W., Associate Professor of Zoology, Columbia University.
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RENN, CHARLES E., Associate Sanitary Biologist, Massachusetts Department of Health.
REYNOLDS, J. PAUL, Professor of Biology, Birmingham Southern College.
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SCHAEFFER, A. A., Professor of Biology, Temple University.
SCHALLEK, WILLIAM B., Teaching Fellow, Harvard University.
SCHARRER, ERNST A., Assistant Professor of Anatomy, Western Reserve University School of Medicine.
SCHMITT, FRANCIS O., Professor of Biology, Massachusetts Institute of Technology.
SCOTT, SISTER FLORENCE MARIE, Professor of Biology, Seton Hill College.
SHAPIRO, HARRY H., Assistant Professor, Department of Anatomy, College of Physicians and Surgeons, Columbia University.
SICHEL, F. J., Associate Professor of Physiology, University of Vermont, College of Medicine.
STEINBACH, H. B., Associate Professor of Zoology, Washington University.
STEWART, DOROTHY R., Research Fellow in Physiology, University of Pennsylvania.
STOKEY, ALMA G., Professor Emeritus of Plant Science, Mount Holyoke College.
STUNKARD, HORACE W., Professor of Biology, Head of Department, New York University.
TAFT, CHARLES H., Associate Professor of Pharmacology, Medical Branch, University of Texas.
TAYLOR, WILLIAM RANDOLPH, Professor of Botany, University of Michigan.
TEWINKEL, LOIS E., Associate Professor of Zoology, Smith College.
TROEDSSON, PAULINE H., Instructor in Biology, Brooklyn College.
WAINIO, WALTER W., Assistant Professor of Physiology, New York University.
WATTERSON, RAY L., Assistant Professor of Zoology, University of California.
WENRICH, D. H., Professor of Zoology, University of Pennsylvania.
WHITING, ANNA R., Associate Professor of Zoology, Swarthmore College.
WHITING, P. W., Associate Professor of Zoology, University of Pennsylvania.
WICHTERMAN, RALPH, Assistant Professor, Temple University.
WITKUS, ELEANOR RUTH, Instructor in Botany and Bacteriology, Fordham University.
WRINCH, DOROTHY, Lecturer in Physics, Smith College.
YNTEMA, CHESTER L., Assistant Professor of Anatomy, Cornell University, Medical College.
ZWEIFACH, BENJAMIN W., Research Associate in Biology, New York University.

Beginning Investigators, 1944

EDELMAN, ABRAHAM, Graduate Student, Columbia University.
HERMANSON, VIRGINIA, Graduate Student, Ohio State University.
JAEGER, LUCENA, Graduate Student, Columbia University.
KEISTER, MARGARET L., Instructor, Wheaton College.
KOOPMAN, KARL F., Graduate Student, Columbia University.
KRUGELIS, EDITH J., Research Assistant and Graduate Student, Columbia University.
LEHMAN, GENE, Teaching Fellow, University of North Carolina.
LOW, RUTH H., Graduate Student, Zoology Department, Columbia University.
MORTENSEN, EDITH, Assistant Professor of Zoology, The George Washington University.

SCHNEYER, LEON H., Instructor, New York University College of Dentistry.
SISSELMAN, CHARLOTTE B., Research Student, Columbia University.

Library Readers, 1944

AMBERSON, WILLIAM R., Professor of Physiology, University of Maryland.
ANDERSON, THOMAS F., Associate, Johnson Foundation, University of Pennsylvania.
ARMSTRONG, PHILIP B., Professor of Anatomy, College of Medicine, Syracuse University.
BEVELANDER, GERRIT, Associate Professor of Anatomy, New York University.
BISSONNETTE, T. HUME, Professor of Biology, Trinity College.
BODIAN, DAVID, Associate in Epidemiology, Johns Hopkins University.
CAHEN, RAYMOND L., Research Assistant, Yale University, Medical School.
CASSIDY, HAROLD G., Assistant Professor of Chemistry, Yale University.
CHOUCROUN, NINE, Cornell University Medical College.
CLARKE, ROBERT W., Research Assistant, Yale University, Medical School.
COX, EDWARD H., Professor of Chemistry, Swarthmore College.
DISCHE, ZACHARIOUS, Department of Biochemistry, College of Physicians and Surgeons, Columbia University.
FAUST, ERNEST C., Professor of Parasitology and Director Department of Tropical Medicine, Tulane University.
FRIDEMANN, ULRICH, Chief of Division of Bacteriology, Brooklyn Jewish Hospital.
FURTH, JACOB, Associate Professor of Pathology, Cornell University Medical College.
GATES, R. RUGGLES, Emeritus Professor, University of London.
GOODRICH, H. B., Professor of Biology, Wesleyan University.
GUREWICH, VLADIMIR, Assistant Visiting Physician, New York College of Medicine and Bellevue Hospital.
HAYWOOD, CHARLOTTE, Professor of Physiology, Mount Holyoke College.
JOHLIN, J. M., Associate Professor of Biochemistry, Vanderbilt University.
KELLER, RUDOLPH, Prague, Czechoslovakia.
KEYES, DONALD B., Professor of Chemical Engineering, University of Illinois.
KRASNOW, FRANCES, Head of Department of Biological Chemistry-Related Basic Sciences, Guggenheim Dental Clinic.
LANDIS, EUGENE M., Professor of Physiology, Harvard University, Medical School.
LEE, RICHARD E., Research Assistant, Columbia University, College of Physicians and Surgeons.
LOEWI, OTTO, Research Professor, New York University.
MAVOR, JAMES W., Provessor of Biology, Union College.
MENKIN, VALY, (Fellow), Guggenheim Research Foundation.
MEYERHOF, OTTO, Research Professor of Biochemistry, University of Pennsylvania.
MOLDAVER, JOSEPH, Research Associate in Neurology, Columbia University.
MOSCHOWITZ, ELI, Assistant Professor of Chemical Medicine, Columbia University.
O'BRIEN, JOHN A., JR., Instructor in Biology, Catholic University of America.
OCHOA, SEVERO, Research Associate in Medicine, New York University, College of Medicine.
OSEASOHN, ROBERT, Student, Long Island College of Medicine.
PERLMANM, GERTRUDE E., Research Fellow, Harvard University, Medical School.
POWDERMAKER, HORTENSE, Assistant Professor of Anthropology, Queens College.
RUNNER, MEREDITH, Instructor, University of Connecticut.
SHEN, SHIH-CHANG, Member, National Institute of Physiology, China.
SHWARTZMAN, GREGORY, Head Bacteriologist, The Mount Sinai Hospital.
SINGER, MARCUS, Harvard University, Medical School.
SMELSER, GEORGE K., Assistant Professor of Anatomy, Columbia University.
SPEIDEL, CARL C., Professor of Anatomy, University of Virginia.
STERN, KURT G., Chief Research Chemist, Overly Biochemical Research Foundation, New York City.
TASHIRO, KIYOSHI, University of Cincinnati, College of Medicine.
WAGNER, CARROLL E., Research Assistant, Histology, Naval Medical Research Institute.
WEIDENREICH, FRANZ, Honorary Director, Cenozoic Research Laboratory, China.
WEINER, NATHAN, Director of Research, Endo Products, Inc.

WILLIER, B. H., Professor of Zoology, Director of Biological Laboratories, The Johns Hopkins University.
WOODWARD, ALVALYN E., Assistant Professor, University of Michigan.
ZORZOLI, ANITA, Teaching Fellow, New York University.

Research Assistants, 1944

ABRAMSKY, TESSIE, Technician, Rockefeller Institute for Medical Research.
BRUNELLI, ELEANOR L., Research Assistant, New York University Dental School.
DEFALCO, ROSE H., Research Assistant-Secretary, University of Pennsylvania.
DZIORNEY, LEON, Research Assistant, New York University.
FRUMIN, M. R., Research Assistant, University of Pennsylvania.
GOLDIS, BERNICE R., Research Assistant, University of Pennsylvania.
GREGG, JOHN R., Graduate Student, Princeton University.
HIRST, SHIRLEY M., Research Assistant, University of Pennsylvania.
HONEGGER, CAROL, Temple University.
HOPKINS, AMOS, Junior Engineering Aide, Massachusetts State Health Department.
LAWLER, H. CLAIRE, Research Assistant, New York University.
LEFEVRE, LINDA, Research Assistant, University of Pennsylvania.
LEFEVRE, PAUL G., Research Assistant, University of Pennsylvania.
LEVY, BETTY, Laboratory Technician, Rockefeller Institute.
MARKS, MILDRED H., Assistant Research Worker, University of Pennsylvania.
MORTON, JANE W., Technical Assistant in Zoology, University of Pennsylvania.
PRICE, WINSTON HARVEY, Research Assistant, University of Pennsylvania.
QUINN, GERTRUDE P., Research Assistant, New York University.
WILSON, WALTER L., Research Associate, University of Pennsylvania.
WOODWARD, ARTHUR A., Research Assistant, University of Pennsylvania.

Students, 1944

BOTANY

CHEW, ROBERT M., Student, Washington & Jefferson College.
DEVINE, VERONA, Student, Hunter College.
GUZMAN, JULIA, Student, Washington University.
HOSKINS, BARBARA, Student, Wellesley College.
MITTLACHER, HELEN, Student, Wheaton College.

EMBRYOLOGY

ANDERSON, JOAN C., Student, McGill University.
COURANT, GERTRUDE E., Student, Swarthmore, College.
CULLEN, SISTER MARY URBAN, Graduate Student, Yale University.
DAVIDSON, MARGARET E. M., Student, McGill University.
FARFANTE, ISABEL PEREZ, Student, Cambridge, Massachusetts.
FINKELSTEIN, GRACE, Teaching Fellowship, New York University.
GETZ, CHARLOTTE E., Undergraduate Student, University of Chicago.
GODWIN, DORIS RUTH, Graduate Assistant, University of North Carolina.
HENLEY, CATHERINE, Graduate Assistant, University of North Carolina.
HONEGGER, CAROL MARIE, Student, Temple University.
KELLEY, ELLEN MARY, Student, New Jersey College for Women.
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ROTH, OWEN H., Instructor in Biology, St. Vincent College.

SCHNELLER, SISTER MARY BEATRICE, Professor, Saint Joseph College for Women.
STRONG, HELEN MARGARET, Teaching Fellow, Smith College.
VISHNIAC, WOLF, Student, Brooklyn College.
WILLIAMSON, FRANCES ALICE, Student, New Jersey College for Women.
WILLIS, MARIAN, Student, Iowa State College.

PHYSIOLOGY

BERNSTEIN, JEANE, Graduate Student, New York University.
CARSON, GWENETH, Student, University of Toronto.
CREGAR, MARY, Demonstrator in Physiology, Bryn Mawr College.
KEISTER, MARGARET LOUISE, Instructor in Zoology, Wheaton College.
MCLEAN, DOROTHY JUANITA, Graduate Student, University of Toronto.
PARTRIDGE, JUDITH ANN, Assistant in Physiology, Vassar College.
PEPPER, BILLIE BARBARA, Student, Radcliffe College.
REICH, EVA, Student, Barnard College.
TAYLOR, BABETTE, Student, Washington University.
THERIEN, MERCEDES, Assistant Research, Montreal University.

ZOOLOGY

AUSTIN, JANE, Student, Randolph-Macon Woman's College.
BANKS, MARY ELIZABETH, Research Assistant, Washington University.
BARROWS, SHIRLEY LOUISE, Student, University of Rochester.
BENSON, ELEANORE BIE, Student, University of Pennsylvania.
BUTT, FERDINAND H., Instructor, Cornell University.
CALKINS, JANET ELIZABETH MORSE, University of Chicago.
CONRAVEY, JUNE ROSE, Student Assistant, Newcomb College, Tulane University.
DEREVERE, JOAN BROOKS, Undergraduate Student, Wilson College.
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DUNBAR, SALLY, De Pauw University.
FAIRFIELD, JANET, Student, Russell Sage College.
FALKNER, ETTA, Instructor, American Museum of Natural History.
FOGERSON, VIRGINIA LEE, Drury College.
GOSFORD, BARBARA, Duke University.
HABERT, YVONNE A., High School Teacher, City of Boston.
KOOPMAN, KARL FRIEDRICH, Graduate Student, Columbia University.
LANGMAN, IDA K., University of Pennsylvania.
LAUTHERS, ROSEMARY ANN, Student, Oberlin College.
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MCCLINTOCK, MARY, Instructor, Bemidji, Minnesota.
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RANDALL, NANCY LOIS, Student, Swarthmore College.
REESE, JEAN, Goucher College.
ROOT, OSCAR M., Instructor, Brooks School.
ROTH, OWEN HAROLD, Instructor in Biology, St. Vincent College.
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SLAVIN, ALICE CECILIA, Student, Seton Hill College.
SOUTHWELL, VIOLET M., Student, Wilson College.
STEENBURG, ISABELLA, Student, Vassar College.
STEKL, ELEANOR B., Science Teacher, N. Tonawanda High School.
SWEENEY, PATRICIA GEORGIA, Student, Oberlin College.
VAN GEYT, VIRGINIA, Student, University of Rochester.
VIOSCA, MIRIAM A., Student Assistant, Newcomb College, Tulane University.
WELLER, DORIS A., Undergraduate, Radcliffe College.
WARNER, ROSE ELLA, Teacher of Biology, Frick Educational Commission.

6. TABULAR VIEW OF ATTENDANCE

	1940	*1941*	*1942*	*1943*	*1944*
INVESTIGATORS—Total	386	337	201	160	193
Independent	253	197	132	89	112
Under instruction	62	59	16	19	11
Library readers	—	31	28	35	50
Research assistants	71	50	25	17	20
STUDENTS—Total	128	131	74	68	75
Zoology	55	55	36	47	37
Protozoology (not given after 1940)	7	—	—	—	—
Embryology	34	37	24	13	23
Physiology	22	24	6	8	10
Botany	10	15	8	—	5
TOTAL ATTENDANCE	514	468	275	228	276
Less persons registered as both students and investigators	7	7	2	6	1
	507	461	273	222	275
INSTITUTIONS REPRESENTED—Total	148	144	126	116	106
By investigators	112	102	83	70	74
By students	79	72	43	41	41
SCHOOLS AND ACADEMIES REPRESENTED					
By investigators	1	5	2	2	1
By students	2	2	—	1	2
FOREIGN INSTITUTIONS REPRESENTED					
By investigators	2	3	—	2	2
By students	1	1	—	—	3

7. SUBSCRIBING AND COOPERATING INSTITUTIONS

1944

Amherst College
Barnard College
Bowdoin College
Brooklyn College
Bryn Mawr College
Catholic University of America
Columbia University
Cornell University
Cornell University Medical College
Duke University
Fish and Wild Life Service, U. S. Dept. of the Interior
Fordham University
Henry C. Frick Educational Commission
Goucher College
Harvard University
Hunter College
Industrial and Engineering Chemistry, of the American Chemical Society
Johns Hopkins University
The Lankenau Hospital Research Institute
Eli Lilly and Co.
Massachusetts Department of Health
Massachusetts Institute of Technology
Mount Holyoke College
H. Sophie Newcomb College
New York University
New York University College of Medicine
New York University Washington Square College
Oberlin College
Ohio State University
Princeton University
Radcliffe College
Rockefeller Institute for Medical Research
Russell Sage College
St. Joseph College for Women
Smith College
State University of Iowa
Syracuse University
Syracuse University Medical School
Temple University
Tufts College
University of Chicago
University of Cincinnati
University of Illinois
University of Maryland Medical School
University of Pennsylvania
University of Pennsylvania School of Medicine
University of Rochester

Vassar College
Villanova College
Washington University
Wayne University
Wellesley College
Wesleyan University
Western Reserve University
Wheaton College
Wilson College
Wistar Institute
Woods Hole Oceanographic Institution
Yale University

8. EVENING LECTURES, 1944

Friday, June 30
Dr. T. H. Bissonnette ..... "Some Recent Studies on Photoperiodicity in Animals, particularly Fur-bearers."

Friday, July 7
Dr. Ethel Browne Harvey ..... "Some Results of Centrifuging the Arbacia Egg."

Friday, July 14
Dr. A. C. Giese ..... "Ultraviolet Radiations and the Life Activities of Cells."

Friday, July 21
Dr. H. J. Muller ..... "Evidence for the Meticulousness of Adaptation."

Friday, July 28
Dr. Carl C. Speidel ..... "Experimental Studies of Special Sensory Organs and Nerves."

Thursday, August 3
Dr. Ernest Carroll Faust ..... "Problems of Tropical Medicine in the United States."

Friday, August 4
Dr. A. K. Parpart ..... "Blood Preservation: A Problem in Cellular Physiology."

Thursday, August 10
Mr. G. G. Lower ..... "Local Invertebrates."

Friday, August 11
Dr. W. C. Allee ..... "Social Orders Among Vertebrates."

Wednesday, August 16
Dr. Ralph Turner ..... "Rehabilitation of Scientific Institutions in Devastated Europe."

Friday, August 18
Dr. A. W. Pollister ..... "The Centriole Problem."

Friday, August 25
Prof. G. H. Parker ..... "Animal Coloration, Fixed and Changeable."

9. SHORTER SCIENTIFIC PAPERS, 1944

Tuesday, July 18
Dr. B. H. Willier ..... "Melanophore Control of Sexual Dimorphism in Feather Pigmentation of the Barred Rock Fowl."
Dr. Viktor Hamburger ..... "The Effects of Peripheral Factors on Motor Neuron Differentiation in the Chick Embryo."
Dr. W. H. Lewis ..... "The Superficial Gel Layer and Its Role in Development."

Tuesday, July 25

Dr. Leonor Michaelis ..... "Ferritin and Iron Metabolism."
Dr. Arnold Lazarow ..... "The Chemical Organization of the Cytoplasm of the Liver Cell."
Dr. Leonor Michaelis ..... "Theory of Metachromatic Staining."

Tuesday, August 1

Dr. Dorothy Wrinch ..... "The Native Protein in Crystalline Form."
Dr. Otto Myerhof ..... "The Role of Adenylpyro-Phosphatase in Alcoholic Fermentation of Yeast."
Dr. Ernest Scharrer ..... "The Naples Station Still Lives."

Tuesday, August 8

Dr. A. M. Shanes ..... "Application of Bio-electricity to the Study of Functioning in Nerve."
Dr. David Nachmansohn ..... "On the Energy Source of the Nerve Action Potential."
Dr. T. H. Bullock ..... "Oscillographic Studies on the Giant Nerve Fiber System in Lumbricus."
Dr. Paul Weiss ..... "Evidence for the Perpetual Proximo-distal Growth of Nerve Fibers."

Tuesday, August 15

Dr. L. V. Heilbrunn ..... "A Toxic Substance from Protoplasm."
Dr. D. L. Harris
Dr. P. G. LeFevre
Dr. W. H. Price
Dr. W. L. Wilson
Dr. A. A. Woodward, Jr.
Dr. D. L. Harris ..... "The Chemical Nature of a Toxic Substance from Protoplasm."
Dr. W. H. Price
Dr. L. V. Heilbrunn
Dr. G. I. Lavin ..... "Recent Developments in Ultraviolet Microscopy."

Tuesday, August 22

Dr. B. W. Zweifach ..... "The Peripheral Circulation in Traumatic Shock."
Dr. W. R. Amberson ..... "Recent Experience with Hemoglobin-saline Solutions."
Dr. R. G. Abell ..... "Gelatin as a Plasma Substitute."
Dr. W. M. Parkins

Thursday, August 24

Dr. C. A. Berger ..... "Experimental Studies on the Cytology of Allium."
Dr. Valy Menkin ..... "Studies on the Chemical Basis of Fever."
Dr. Miriam F. Menkin ..... "In Vitro Fertilization of Human Ova."

Tuesday, August 29

Dr. L. M. Bertholf ..... "Studies on Metamorphosis in the Tunicate."
E. Mortensen ..... "Behavior and Tube Building Habits of Polydora ligni."
Dr. P. S. Galtsoff
Dr. J. B. Buck ..... "The Click Mechanism of Elaterid Beetles."

Thursday, August 31

DR. G. K. SMELSER ..... "Orbital Changes in Experimental Exophthalmos."

DR. A. GORBMAN ..... "Radioactive Iodine Absorption in Lower Chordates and the Problem of Homology of the Thyroid Gland."

DR. D. L. HARRIS ..... "Phosphoprotein Phosphatase, a New Enzyme from the Frog Egg."

10. MEMBERS OF THE CORPORATION, 1944

1. LIFE MEMBERS

ALLIS, MR. E. P., JR., Palais Carnoles, Menton, France.
ANDREWS, MRS. GWENDOLEN FOULKE, Baltimore, Maryland.
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HÖBER, DR. RUDOLF, University of Pennsylvania, Philadelphia, Pennsylvania.
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DOMINANT LETHALITY AND CORRELATED CHROMOSOME EFFECTS IN HABROBRACON EGGS X-RAYED IN DIPLOTENE AND IN LATE METAPHASE I [1]

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Introduction

If oviposition is prevented in well-fed females of the parasitic wasp Habrobracon by witholding them from their host they continue to produce mature eggs until the egg sacs are filled. These stored eggs may number as many as twenty per female and are in late metaphase of the first meiotic division (metaphase I). Their retention in this stage for four days has no effect on their hatchability which is 96 per cent in the wild type stock used for the experiments herein described.

When unmated females with stored eggs are x-rayed and allowed to oviposit at 30° C. all eggs laid during the first six hours after treatment will have been irradiated in late metaphase I. Eggs laid during the seventh and eighth hours after treatment consist of a variable mixture treated in metaphase I and in late diplotene (including all eggs in diakinesis) and are, therefore, of no use in the present study. Eggs laid during the ninth to twelfth hours after treatment will have been post-synaptic with their diffuse chromosomes in a relatively quiescent condition when irradiated. These are designated as late and early diplotene.

An advantage in the use of these eggs for the detection of injuries lies in the fact that they develop parthenogenetically if unfertilized and so indicate directly the effects of treatment on a haploid set of chromosomes. Disadvantages are the large number ($n = 10$) and small size (less than 1 μ in diameter) of their chromosomes. The details of oogenesis appear to be orthodox and so the results should be universally applicable to forms with comparable type of meiosis. Failure to hatch and cytological changes in stages immediately following treatment have been the criteria of injury. Preliminary results were first published in 1938 (Whiting, 1938). Details of technique and hatchability effects, as well as extensive bibliography, are given elsewhere (Whiting, 1945); cytological effects and their correlation with mortality and dose are presented here in detail.

Dose-hatchability Relationships

Hatchability effects may be summarized briefly. No correction for control hatchability is made since it is so close to 100 per cent. Eggs x-rayed in diplotene

[1] This investigation has been aided by a grant (to P. W. Whiting) from the Rockefeller Foundation, for apparatus and technical assistance. The work was done at the Zoological Laboratory of the University of Pennsylvania and at the Marine Biological Laboratory, Woods Hole, Massachusetts. To these institutions the author is grateful for the use of laboratory facilities and of x-ray equipment. Valuable assistance was also given by the American Oncologic Hospital of Philadelphia through the use of x-ray equipment.

and allowed to develop parthenogenetically give a dose-hatchability curve which appears to be linear at low doses and to become "mixed" at high doses; they have 50 per cent mortality at 12,000 *r* and 100 per cent at about 45,000 *r*; they showed no significant change in hatchability in preliminary and inadequate tests of time-intensity differences. Those treated in early diplotene (laid during the eleventh and twelfth hours after treatment) show no change in hatchability at any dose with fractionated treatment, those treated in late diplotene (laid during the ninth and tenth hours after treatment) show a significant increase at high doses with fractionated treatment. The dose-hatchability curve for combined diplotene owes much of its mixed character at high doses to late diplotene, early diplotene response being more nearly linear.

Eggs x-rayed in late metaphase I and allowed to develop parthenogenetically show a linear decline in hatchability with increasing dose and have 50 per cent mortality at 375 *r*, 100 per cent at about 1,400 *r*; they show no change in dose-hatchability relationships with aging between treatment and oviposition, time-intensity differences or fractionation of dose.

When Habrobracon females are mated, about two-thirds of the eggs are fertilized. If treated females are mated to untreated males, the survival of any appreciable number of eggs through the aid of normal spermatozoa would increase percentage of hatchability thereby indicating the presence of recessive lethal effects by comparison with hatchability of eggs from treated unmated females. Table I

TABLE I

Hatchability percentages for eggs of treated females, unmated and mated to untreated males

Stage treated	Dose in *r* units	Unmated females		Mated females	
		Number of eggs	Hatchability percentage	Number of eggs	Hatchability percentage
Metaphase I	560	319	39.8±2.7	318	40.5±2.7
Prophase I	5,600	137	71.5±2.6	126	70.6±4.0
	22,400	100	19.0±3.9	182	19.2±2.9
Controls	0	127	98.4±1.1	363	98.6±0.6

demonstrates that most, if not all, of the lethal effects induced in these stages by x-rays are dominant, at least in respect to hatchability. This is rather surprising at first glance but in treated metaphase I, as pointed out below, chromosomal deletions appear to be relatively large and in either stage, it is possible that deletions small enough to act as recessives in fertilized eggs may not kill the individual until after hatching in unfertilized eggs. Lethals which are recessive in diploids may be due to such minute losses as to exert their effects only after hatching in haploids. Perhaps viable deficiency heterozygotes are so rare that hatchability of irradiated eggs is not perceptibly altered by fertilization with untreated spermatozoa. In any case, conditions are well suited to an analysis of dominant lethal ratios induced by x-rays in identifiable stages of meiosis and, although the chromosomes present

difficulties, the eggs themselves are easily handled, fixed and stained for observation.

About 40,000 eggs were collected and observed for hatchability. Records were kept of the results from individual females in all cases so that aberrant behavior in eggs from any individual could be recognized. Such behavior was extremely rare.

From the work of Sax (1938, 1940), Fabergé (1940) and others on dose-chromosome injury curves, certain tentative conclusions were drawn concerning cytological effects before study of chromosomes was begun. For diplotene it was assumed that the great majority of chromosome breaks must undergo restitution; that broken ends of chromosomes within the same cell increase as dose increases, permitting complicated reunions (translocations, large interstitial deletions) so that lethal individual chromosome changes tend to be due increasingly to more than one ionization, especially in late diplotene; that bridges can be formed in either meiotic division or in both, due to lateral fusion of the broken ends of chromatids whenever two adjoining chromatids are broken by a single ionization. From the work of Sturtevant and Beadle (1936) and of McClintock (1941) it was thought that bridges in division I might be permanent or delayed in breakage and might offer an explanation for some, at least, of the high resistance of this stage to irradiation.

Concerning metaphase I it was assumed, because of the linear relationship of hatchability to dose, that injuries were in the form of terminal deletions or of minute interstitial deletions, in other words, injuries due to single ionizations. The high sensitivity of this stage suggested that most injuries must be permanent. It was doubted that a single ionization would break two chromatids due to the degree of separation in late metaphase I and so the occurrence of bridges in either division from this cause seemed improbable. There was also the possibility that high metaphase sensitivity might be due to "physiological" effects, stickiness, etc., which would result in fusion bridges, delay in division or death.

Cytological Observations

Cytology of controls. The cytology of the stages before metaphase I has not been studied in detail, either in control or irradiated material, because of the small size and large numbers of chromosomes and of their elongate and diffuse condition. Synapsis occurs in very young oocytes and the subsequent behavior through condensation appears to have nothing exceptional about it. Changes take place slowly and are not obvious in character until just before condensation of chromosomes (diakinesis) when tetrads move to periphery of the nucleus. Most students of hymenopteran cytology would question the conclusion that the stored oocyte is in an orthodox and identifiable stage, late metaphase I. They state that the chromatin has reverted to a resting stage or has formed an abortive spindle, a compact clump or a composite body, etc. Speicher (1936) finds that the most advanced eggs in the Habrobracon egg sac are in "early anaphase of the first maturation" which the author prefers to call late metaphase. Speicher's observations that distinct chromosomes are present, are in the form of tetrads (Fig. 1) and are ten in number has been repeatedly checked by the author and cannot be questioned. They show the

forms expected for tetrads and each resolves immediately into two pairs of dyads upon completing division I. The conclusion must be drawn either that Habrobracon differs from many other Hymenoptera in having orthodox oogenesis or that its chromosomes retain more easily their individuality when fixed.

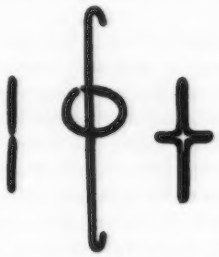

FIGURE 1. Three tetrads from one late metaphase I spindle. Untreated. Drawn from whole mount of egg with aid of a camera lucida. Semi-diagrammatic. × 4,625.

The stages of normal oogenesis following oviposition, as described by Speicher, are briefly as follows. During the process of oviposition the maturation spindle is moved from dorsal to ventral side of the egg. It then passes into telophase I. The second division follows immediately. The four groups of chromosomes (1a, 1b, 2a, 2b) lie in a row roughly perpendicular to the egg surface. During anaphase II polar nuclei 1a and 2a remain stationary, 1b moves close to 2a, and 2b (functional nucleus) sinks deeper into the egg, a membrane forming as it moves. Nucleus 1a soon disintegrates, 1b and 2a unite and form a metaphase plate which divides and then disintegrates. Cleavage is of the usual insect type, with nuclei moving about until blastoderm formation when cell membranes first appear. The stages following oviposition are the ones which were studied after irradiation.

No evidences of displaced chromosomes or of aberrant conditions resembling those observed in irradiated eggs were found by Speicher or by the author in large numbers of control eggs studied.

Cytology of irradiated eggs. In experiments concerned with cytological effects, eggs from control and treated females were incubated according to standard schedules, dropped into fixative (formalin-acetic-alcohol), punctured at the posterior end to facilitate fixation, treated with the Feulgen technique and mounted whole in balsam. Control hatchability tests were made of eggs treated at the same time as those fixed. Slides were made of about 2,500 eggs.

After treatment in diplotene acentric fragments, dicentrics or both may occur in division I (Fig. 2a, b, c) or in division II or in both divisions. Bridges in division I may be permanent and can be seen bulging at the side when nucleus 1b moves towards 2a, indicating that chromatin bridges are tensile but not elastic (Fig. 2c). Acentric fragments remain visible throughout both divisions. No evidences of stickiness or of clumping of chromatin (Fig. 2a, b, c) or of retardation of meiosis are apparent for doses up to lethal (45,000 *r*). Of eggs treated in diplotene with 44,800 *r*, 1.1 per cent died at first cleavage, 30.4 per cent with a few nuclei, 54.3 per cent with many nuclei and 14.2 per cent at blastoderm.

Immediately after irradiation in late metaphase I (Fig. 1), chromosomes show no apparent change but at telophase I acentric fragments are left within the spindle and these remain visible throughout division II (Fig. 2d, e). They are often almost as large as entire chromosomes and can usually be identified as double structures. No bridges have been seen in division I in over 1,500 eggs observed. In

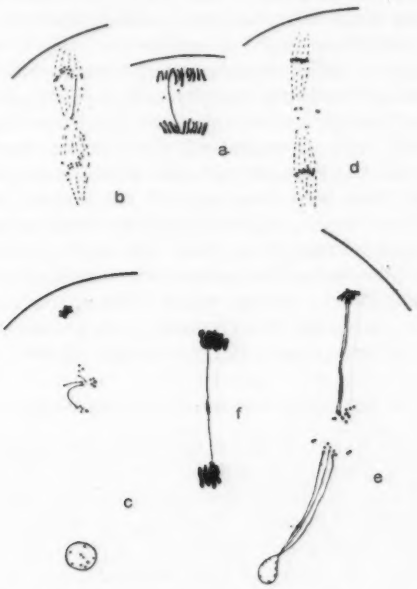

FIGURE 2. Illustrations were drawn from whole mounts of eggs with the aid of a camera lucida. *a*, *b*, and *c*, eggs were irradiated in late diplotene with 44,800 *r*; *a*, telophase I. × 1,500, *b*, metaphase II. × 875; *c*, telophase II. × 1,250; *d*, *e*, and *f*, eggs were irradiated in late metaphase I with 2,000 *r*; *d*, metaphase II. × 875; *e*, telophase II. × 1,250; *f*, third cleavage, telophase. × 4,550.

division II bridges occur and after heavy treatment (2,000 *r*) several may be seen in each second division spindle (Fig. 2e). Small fragments occasionally appear in division II spindles. No evidences of stickiness or of clumping (Fig. 2d, e) or of retardation of meiosis occur in development following treatment with lethal dose of this stage which, except for absence of bridges in division I, behaves cytologically as treated diplotene. Percentages of eggs with fragments in division I and mean number of fragments per treated increase linearly with increased dose (Whiting, 1945). All eggs exposed in late metaphase I to 2,016 *r* undergo some development. 2.4 per cent die in first cleavage, 7.2 per cent with a few nuclei, 71.4 per cent with many nuclei and 19.0 per cent at blastoderm stage. In spite of their high sensitivity, some eggs treated in metaphase I developed to the fifth cleavage (expected) after 15,000 *r*, to metaphase II after 25,000 *r*, to pronucleus after 35,000 *r* and one to anaphase II after 200,000 *r*. No records were kept of rate of development at these higher doses.

The similar patterns of stage at death for both diplotene and metaphase I at their respective lethal doses indicate that, in spite of the great difference in sensitivity between the stages, cause of death is of the same nature in both. These data on time of death check what has often been noted, especially in respect to mature spermatozoa, that so-called lethal doses are not actually lethal to the treated cell itself but, instead, to its descendents. The fact that the oocyte continues to function normally and that death does not occur until it becomes an embryo, supports the argument that cytoplasmic injury is not at the basis of mortality. It is due, rather, to loss of parts of chromosomes during meiosis following irradiation and to resulting incomplete chromosome complements in every cleavage nucleus.

Bridges occur in cleavage I after treatment in either stage indicating that, if chromatids are already split when treated, there occurs lateral fusion of broken ends of half-chromatids. If they are not split when treated the split occurring in the first mitosis must have been incomplete in the broken chromatid or have resulted in lateral fusion of broken ends of daughter chromosomes. Bridges appear in subsequent cleavages. Fragments, which also occur in cleavage, could not be explained at first until it was noted that they are tapering at the ends and that they result from double breaks in a bridge which releases a thickened middle portion (Fig. 2f). Fragments were not observed after every mitosis in the same embryo although bridges, if present, appear in all cleavage figures.

Correlation of Injury with Chromosome Form when Treated

It is perhaps unwise to devote much time and space to the subject of the correlation of the nature of the injuries and the form of the chromosomes when treated in view of the small size of Habrobracon chromosomes and the disagreement of investigators in this field. Obviously, there is a correlation. The studies of Sax (1938, 1940), Fabergé (1940) and McClintock (1938) will be used as a basis of a brief discussion, since the results of these investigations are consistent with their theories.

Chromosome injuries fall into two classes, those caused by single ionizations and those caused by more than one. The former consist of terminal deletions and minute interstitial deletions. Two identical terminal deletions can be induced by a single ionization if two chromatids are sufficiently close together. When this happens, lateral fusion of broken ends occurs resulting in a dicentric, from parts of the two chromatids still attached to spindle fibers, and an acentric, from the released and fused ends. Single terminal deletions can be induced by single ionizations and this appears to be the rule when chromatids are widely separated. An acentric is ultimately lost and a dicentric forms a bridge when its two spindle fiber attachment points (centromeres) are pulled apart. If the bridge does not break, an entire chromosome may be missing from a daughter cell. If it does break, the resulting chromosomes are incomplete and each daughter cell will have an incomplete chromosome and, therefore, an incomplete set of genes. Such a terminally incomplete chromosome may continue to form a bridge in each subsequent division, either by failing to split completely or by a lateral fusion of the broken ends after splitting. This appears to be the general rule but McClintock (1941) has found that when such an incomplete chromosome occurs in the sporophyte tissue of maize, it forms no bridge.

An interstitial deletion caused by a single ionization in a chromosome would mean the loss of genes and would be lethal if they were numerous or of sufficient importance but it would not be cytologically apparent in subsequent divisions because of its small size.

Injuries which must be due to more than one ionization since they involve breaks in chromosomes too far apart to be caused by a single ionization are large inversions, large interstitial deletions and translocations. Inversions would not be apparent, either cytologically or in effect on viability of the embryo receiving them in the present study since they would be induced after synapsis and crossing over and the inversion of a block of genes would probably have no lethal effect. Large interstitial deletions would have a lethal effect but could not be identified in material used in these experiments. Translocations might be lethal and would be visible as bridges should centric parts of non-homologues become attached to each other. Such bridges cannot be distinguished cytologically from those resulting from double terminal deletions in this material.

The nature of the hatchability curves suggests that most injuries in early diplotene and late metaphase I at all doses and in late diplotene at low doses are caused by single ionizations, that many injuries in late diplotene at high doses are caused by more than one ionization. Since there is no reason to suppose that the nature of original breaks would be changed by higher doses it is presumed that the number of single breaks per cell increases with high dose and allows greater opportunity for new combinations because of increased number of broken ends available at any one time. This would take for granted the breaking of single chromatids per ionization for if two were broken the lateral fusion of broken ends would prevent translocations, fusion with more distant chromosomes. The reduction in injury by fractionation of dose is explained on the grounds that, with repeated smaller doses, fewer free ends are available at any given time for new combinations and the intervals between treatments afford an opportunity for restitution or changes in broken ends to occur so that they are no longer capable of joining with other broken ends formed by later treatments.

Three conditions seem to be of importance, then, in determining response of the chromosomes here studied to irradiation. These are (1) relation of tetrads to each other in the nucleus, (2) degree of separation of adjoining chromatids within a tetrad and (3) nature and degree of tension on chromosomes. Each of the three stages will be discussed briefly from these points of view.

In early diplotene the tetrads are evenly distributed within the nucleus, sister chromatids are in contact, homologues separated except at chiasmata, and neither traction of the spindle fibers nor terminalization has begun. Most breaks will be temporary because of lack of tension and relaxed state of the chromosomes. Translocations should be possible but the majority of breaks will involve both sister chromatids with the production of acentrics and dicentrics. Permanent double breaks can occur either between centromeres and proximal chiasmata (with production of bridge in division II) or distal to chiasmata (producing bridge in division I if distal to "odd" chiasmata, in division II if distal to "even") since the slight tension which exists is equally exerted everywhere along the length of the chromosome.

In late diplotene the tetrads move peripherally but are still widely separated, terminalization (movement of chiasmata towards ends of tetrads) has begun, as

well as movement of centromeres away from each other, and chromatids are not so closely associated, especially toward ends of chromosomes. Single and double breaks will occur (the latter nearer the centromere) and more of them will be permanent because of new tensions. Bridges should be less frequent in division I than in the case of early diplotene but this has not been checked. This stage will be somewhat more sensitive and will exert its lethal effects through translocations and large interstitial deletions as well as through double terminal deletions.

In late metaphase I, the tetrads are isolated from each other and stable in position on the spindle so that interchanges between them would not be expected. Centromeres are pulled far from each other and chiasmata resist further terminalization (Fig. 1) so that tension exerted between centromeres and proximal chiasmata is very great, tension exerted distal to chiasmata not so great. Ionizations will cause double breaks near centromeres where sister chromatids are closely approximated and these will all be permanent because of the extreme tension. They will result in large double fragments (acentrics) in division I, bridges (dicentrics) in division II. Breaks induced towards ends of chromosomes, and especially distal to chiasmata, will be less likely to be permanent and more likely to be single. There will be few or no bridges in division I and single fragments will appear in division I or division II (McClintock, 1938).

Any injury to a tetrad which results in a single bridge in division II reduces the chance of hatching of the egg by fifty per cent; in division I the effect is the same if the bridge breaks promptly. If it is delayed in breaking or does not break the hatchability of the egg is not affected, since an incomplete chromatid is thereby restrained from entering the ootid nucleus. A single terminal deletion reduces the chance of hatching by twenty-five per cent.

With ten tetrads of the diverse forms found in Habrobracon, combinations of changes induced by single ionizations can become very complex. If added to these are the complication of translocation and of large interstitial deletions (characteristic especially, perhaps, of late diplotene) the great resistance of diplotene is to be wondered at. The author (Whiting, 1945) has reviewed the data here reported in the light of the numerous theories devised to explain differential sensitivity of chromosomes to x-rays and has found that the only one which applies is that put forth by Goodspeed in 1929. He suggested tension as the important factor. It seems highly probable that numerous breaks do occur in the evenly distributed, diffuse, slowly moving chromosomes of diplotene but that the majority of them is temporary. Healing or restitution must take place quickly for there is always some movement and these chromosomes ultimately go through the same stresses as those treated in late metaphase I and, in addition, those attendent upon condensation and complete terminalization. Their response to fractionation also argues for relatively rapid restitution.

The development of individuals, normal in appearance and in reproductive activity and with normal descendents, after treatment in diplotene with 35,000 *r*, illustrates graphically the resistance of this stage to permanent injury by ionizations.

Sax (1942) summarizes the information available on "physiological" effects of x-rays, one of which is stickiness of chromatin. It has been found that condensed chromosomes are most sensitive in respect to stickiness, that such effects are temporary, delay subsequent division, have a threshold dose, are lethal only after

very high doses and result in "fusion" bridges if the cell divides before recovery. The stage in the present study most likely to show the effects of stickiness in the form of fusion bridges is division I after treatment of metaphase I. This is the only division which shows bridges of no kind even after doses much higher than lethal. A delay of twenty-four hours between treatment and resumption of meiosis does not increase hatchability, meiosis is not appreciably delayed after irradiation, there is no threshold effect (down to 50 *r*). It should be emphasized again that, wide apart as are the lethal doses for diplotene and metaphase I, at their respective lethal doses, the pattern of stages at death is the same, the same percentage dies at first cleavage, at blastoderm, etc.; in other words, 45,000 *r* has no more drastic effect on development of treated diplotene than 1,400 *r* on metaphase I. All evidence indicates that cause of death is of the same order for both stages and that "physiological" effects are of every minor importance, and not appreciably different in the two stages.

Sturtevant and Beadle (1936) failed to recover an expected genetic type of chromosome aberration correlated with a dicentric in division I. They suggested that in a form like Drosophila where the four meiotic nuclei lie in a row and where a terminal one alone functions, a bridge in division I might fail to break, or might be delayed in breaking, thereby tying together injured chromatids and allowing uninjured ones to pass to the terminal nuclei. McClintock (1941) also offers as explanation for the failure to obtain expected genetic results correlated with bridges in division I of maize, the selective effect of these bridges on broken chromatids. Terminal nuclei (one of which becomes the functional megaspore) tend to receive the uninjured chromatids. Figure 3c demonstrates that bridges in division I in Habrobracon eggs do not break, at least in some cases.

In divisions following treatment of diplotene to which this selection of injured chromatids for elimination would apply, the chances of having bridges in the second division are as frequent as in the first or more so and selection through permanence of bridges would apply, therefore, only in the simplest kind of injury and that to but one or very few tetrads since any number of breaks would be certain to produce some bridges in division. This selection, although it undoubtedly occurs, cannot explain more than a small amount of resistance of diplotene. It would be expected to apply especially with low doses when but a single break occurs in a single tetrad.

The wide difference in size of lethal doses (45,000 *r*–1,400 *r*) of such closely related stages of the same cell, the unreduced Habrobracon egg, confirms the truth of the conclusion made long ago (1906) by Krause and Ziegler in an extensive and critical study of tissue injury by x-rays, that it is less the kind of cell than its stage at the time of treatment which determines sensitivity.

The facts and theories just presented are of interest in connection with a discussion of dominant lethals by Pontecorvo (1942). He explains dominant lethal effects in Drosophila spermatozoa by assuming that single chromosome breaks are produced by radiations at a rate proportional to radiation dose and that these neither undergo restitution nor participate with other breaks in the same nucleus in rearrangements. "Chromosomes with broken ends give rise to a cycle of breakage-fusion-bridge phenomena in development." He also writes, "It is therefore an open question whether sister unions are so frequent as to cause a considerable portion of dominant lethality. Should this be the case, the trend of the curve of dominant lethality could be explained. Most dominant lethality would be de-

termined by single-break sister unions at low dosages and as the dosage increased lethal changes of the other two types (translocations and deletions) would come to play an increasing part." Translocations and deletions would not be produced actually until syngamy since breaks appear to remain open in the sperm chromosomes until that time.

Broken chromosome ends do undergo restitution or participate with other broken ends in the egg very soon after treatment but the final contribution to the zygote may be the same as that made by the irradiated sperm, viz., a chromosome with a broken end which will give rise to the breakage-fusion-bridge cycle in the first cleavage as well as in subsequent ones. Most dominant lethality in the present study is, without much doubt, caused by single ionizations and only in late diplotene at high doses does treatment appear to cause a high percentage of death from the cooperation of two or more ionizations.

It is of interest in this connection to note that the dose-injury curve for dominant lethality in the spermatozoa of Drosophila (Sonnenblick, 1940; Demerec and Fano, 1944) and for Habrobracon (Heidenthal, 1945) is of the same nature as that for late diplotene.

Conclusion

1. (Tentative) The majority of dominant lethals induced in late diplotene by low doses (to 11,000 *r*) and in early diplotene and in late metaphase I by all doses through lethal, in Habrobracon eggs, is caused by single ionizations which break adjoining chromatids. Lateral fusion of broken ends results, followed by continued breakage-fusion-bridge phenomena in cleavage. With doses above 11,000 *r* in late diplotene an increasing number of lethal changes arises from two or more ionizations (translocations, large interstitial deletions). 2. Lethal doses are not lethal to the treated cell (oocyte) itself but to its descendents (embryo). Fragmentation of chromosomes is not lethal, loss of fragments is. 3. The nature and degree of chromosome injury can be correlated with the form of the chromosome and with forces acting upon it during and immediately following treatment. 4. The kind of cell is less important than its stage in determining sensitivity to x-rays. 5. Tension is the main factor in determining permanence of breaks caused by ionizations, chromosome form and movement in determining the nature of the new combinations of broken ends. 6. The chromosome phenomena here dealt with are common ones in the majority of animals and plants and it is predicted that, when metaphase and anaphase are sufficiently studied in other forms, they will be found to be the stages most sensitive to x-rays. This has proved to be the case for Sciara (Metz and Bozeman, 1940; Reynolds, 1941) and for Trillium (Sparrow, 1944).

Summary

Unlaid Habrobracon eggs x-rayed in diplotene (lethal dose about 45,000 *r*) and allowed to develop parthenogenetically, show fragments, bridges or both in division I; either or both in division II. Bridges in division I may be permanent.

Unlaid eggs x-rayed in late metaphase I (lethal dose about 1,400 *r*) show fragments but no bridges in division I; bridges, fragments or both in division II.

An explanation of difference in cytological effects of x-rays on these stages and of the differences between them in sensitivity and in nature of survival curves is attempted through comparison with studies on forms with larger chromosomes.

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STRATIFICATION AND BREAKING OF THE ARBACIA PUNCTULATA EGG WHEN CENTRIFUGED IN SINGLE SALT SOLUTIONS

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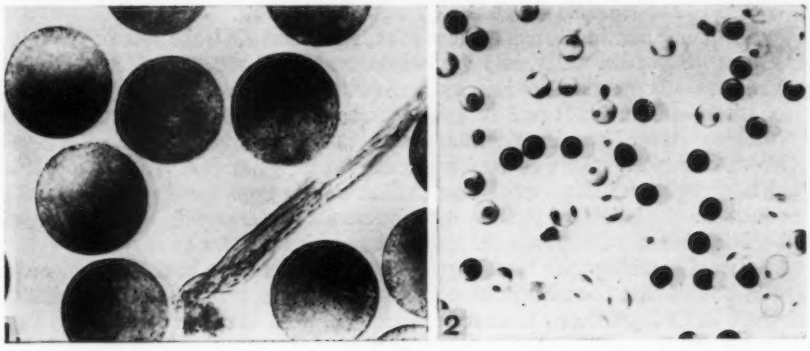
A study has been made of the comparative rate of stratification and breaking of the *Arbacia* egg in single salt solutions, when subjected to centrifugal force. It might be expected that when more rapid stratification occurs, the eggs would break apart more readily. This was, however, found not to be the case when the eggs were centrifuged in hypo- and hypertonic sea water, but this is probably due to the change in volume of the eggs (E. B. Harvey, 1943). With the increased surface area of the eggs in hypotonic sea water the tension at the surface is increased (Cole, 1932) and the eggs are more difficult to break apart. In the present experiments with pure salt solutions the surface area remained constant.

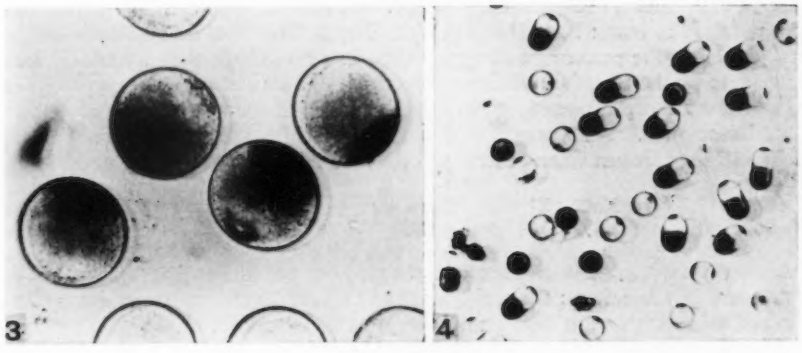
The solutions used in the following experiments were those routinely used at Woods Hole as isotonic with the sea water there, and found by me to be isosmotic on measuring the eggs after immersion, namely: 0.52 m NaCl, 0.53 m KCl, 0.34 m $CaCl_2$, and 0.37 m $MgCl_2$. The pH of the solutions was found to be respectively, 5.54, 5.44, 5.53, and 6.31. It was determined, however, that the pH in itself, at least of sea water, has no effect on the stratification and rate of breaking. Sea water was made up of pH ranging from 5 to 9 by adding HCl or NaOH; eggs kept in these solutions and centrifuged in them at the same time as those in normal sea water showed no difference in stratification or breaking. This was found also by Barth (1929) for stratification in sea water, though he did find an effect in NaCl. However Heilbrunn (1928, 1943) finds that Na definitely increases viscosity. The eggs were not injured by the pure salt solutions as they could be fertilized on removal to sea water after 40 minutes in the solutions and produced normal plutei. However, the eggs cannot be fertilized while in the solutions; the sperm are immotile in all except NaCl, and here no fertilization membrane was seen.

Arbacia punctulata eggs were placed in 50 cc. of the isosmotic salt solution for 20 minutes and this was replaced by a fresh salt solution for another 20 minutes. Three tubes of experimental eggs (in different salt solutions) and one tube of control eggs (in sea water) were centrifuged at the same time; isosmotic sugar solution was placed in the bottom of each tube to keep the eggs suspended. Care must be taken that exactly the same amount of sugar solution is used in each tube and exactly the same amount of egg suspension placed on top, so that the eggs in each tube are thrown to the same level and are subjected to exactly the same amount of centrifugal force. For stratification the force used was about $3{,}000 \times g$ for two minutes, and for breaking $10{,}000 \times g$ for four minutes. Each experiment was repeated many times. A single batch of eggs was always used in each experiment.

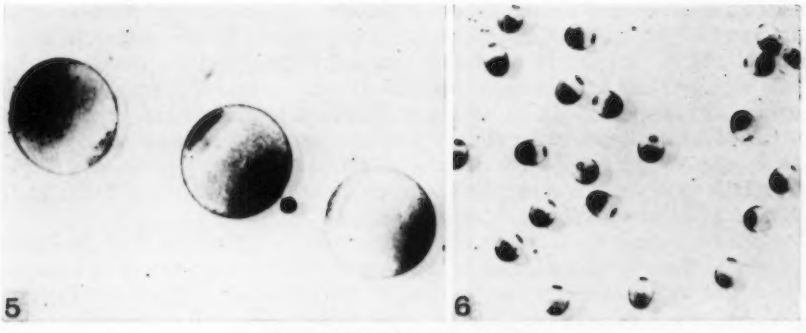
Stratification NaCl (KCl) Breaking



Sea Water



$MgCl_2$ ($CaCl_2$)



EXPLANATION OF PLATE

Stratification of *Arbacia punctulata* eggs centrifuged at 3,000 × g for two minutes in (1) NaCl, (3) sea water, (5) $MgCl_2$. Breaking apart of eggs at 10,000 × g for four minutes in (2) NaCl, (4) sea water, (6) $MgCl_2$. KCl acts much like NaCl and $CaCl_2$ much like $MgCl_2$.

The experiments were carried out at approximately 23° C., so that the temperature effect observed by Costello (1934, 1938) was not involved.

It was found that in the monovalent salts, NaCl and KCl, the rate of stratification is less than in sea water, and in the bivalent salts, $CaCl_2$ and $MgCl_2$, the rate of stratification is greater than in sea water (Photographs 1, 3, 5). The viscosity, then, is increased in NaCl and KCl and decreased in $CaCl_2$ and $MgCl_2$. In the effect on the rate of stratification the series runs, from most to least: Ca > Mg > S.W. > Na > K. This is similar to the series given by Heilbrunn (1923, 1928) in a slightly different experiment with *Arbacia* eggs, except that Na and K are reversed. This is possibly due to a difference in the tonicity of the solutions used. His series for *Stentor* is the same as my series for *Arbacia*.

In ease of breaking with centrifugal force, the series runs in the reverse order. Eggs in KCl, where the stratification is least in a given time, break most readily, and those in $CaCl_2$, where the stratification is greatest, break least readily. Eggs in the monovalent salts, NaCl and KCl, break more readily than those in sea water while the eggs in the bivalent salts, $MgCl_2$ and $CaCl_2$, break less readily than those in sea water (Photographs 2, 4, 6). In ease of breaking, the series runs, from greatest to least: K > Na > S.W. > Mg > Ca. The ease of breaking has been judged by the percentage of eggs broken in a given time with a constant force, rather than by the time for a definite percentage to break, since the experiment can be carried out more accurately when experimental and control eggs are centrifuged at the same time. An average experiment gives the following figures for percentage of eggs broken when centrifuged for four minutes at 10,000 × g.

KCl	NaCl	Sea water	$MgCl_2$	$CaCl_2$
99%	90%	50%	20%	none

There was no measurable difference in the relative size of the two "halves" in any of the pure salt solutions; the white and red "halves" were the same size as those obtained when eggs were kept and centrifuged in sea water.

There is considerable variation in ease of breaking in different lots of eggs with the same centrifugal force, and even the same batch varies slightly after being kept in sea water for several hours. In one experiment 98 per cent were broken in sea water, and 40 per cent in $CaCl_2$; in another experiment, 50 per cent were broken in NaCl and 20 per cent in sea water. In every experiment, however, the eggs in the solutions broke in the order named. It was thought that possibly the jelly surrounding the eggs might be influenced by the salt solutions and be responsible for the difference in ease of breaking. Jelly was found to be present on the eggs in all the solutions. Eggs from which the jelly was removed by addition of 0.2 cc. N/10 HCl to 50 cc. sea water, and then well washed in sea water broke in the solutions in the same order as those with jelly.

Since the experimental results are contrary to the expectation that the interior viscosity is the controlling factor in breaking of the eggs, we are led to the conclusion that the salts affect the "tension at the surface." Despite the increased interior viscosity in pure NaCl and KCl, the surface forces resisting the pulling apart of the eggs are actually decreased. In $CaCl_2$ and $MgCl_2$ they are increased though the interior viscosity is decreased. Heilbrunn (1923, 1943) has pointed out that in *Amoeba*, and apparently also in *Arbacia* eggs, the cortical protoplasm

reacts differently from the interior protoplasm, and Brown (1934) has found a difference in cortical and interior protoplasm in response to hydrostatic pressure on fertilized *Arbacia* eggs.

An effect on the surface forces without any effect on the interior viscosity is given by eggs in Ca-free sea water. Unfertilized eggs kept and centrifuged in Ca-free sea water stratify at the same rate as those in sea water, as shown in previous experiments with a double image centrifuge microscope (E. B. Harvey, 1933). They break apart more readily in Ca-free sea water than in normal sea water—at about the same rate as those in NaCl alone. The fertilized eggs also break more readily in Ca-free sea water than in normal sea water, as shown previously. The absence of calcium therefore tends to decrease the surface forces and the presence of calcium alone tends to increase them. That calcium has an effect on the surface layers of eggs is well known, and has been especially emphasized by Heilbrunn (1928, 1943). A very good example is given by the classic experiments of Herbst (1900) in separating blastomeres due to the dissolution of the ectoplasmic (hyaline plasma) layer in Ca-free sea water.

Summary

When unfertilized *Arbacia punctulata* eggs are centrifuged in isosmotic single salt solutions, they stratify with decreasing readiness (indicating increasing viscosity) in the following order: $CaCl_2 > MgCl_2 >$ S.W. $>$ NaCl $>$ KCl. They break into "halves" with decreasing ease in the reverse order, those in $CaCl_2$ which stratify best, break least readily. In the bivalent salts they stratify better and break less readily than in sea water, and in the monovalent salts they stratify less and break more readily than in sea water. The ease of breaking must be determined by an effect of the salts on the surface layers rather than by their effect on the interior viscosity.

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THE EFFECT OF CYANIDE ON RESPIRATION IN PARAMECIUM CAUDATUM AND PARAMECIUM AURELIA[1]

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In some ciliates the presence of a cytochrome-oxidase system has been established. Pitts (1932) claimed that *Colpidium campylum* showed an ititial sensitivity to HCN but that the oxygen consumption soon increased until it even surpassed normal consumption. Lwoff (1934) also found an initial inhibition followed by an acceleration in respiration in another ciliate, *Glaucoma pyriformis*, when it was exposed to KCN. Hall (1941) definitely established that HCN inhibits respiration in *Colpidium campylum* and Baker and Baumberger (1941) found that HCN inhibits respiration in *Tetrahymena geleii*.

Paramecium is usually cited as one of the several exceptions to the rule that most animal cells are sensitive to HCN. In fact, ciliates as a group have been regarded by some investigators as being insensitive to cyanide, although very few species have been tested. Lund (1918), Shoup and Boykin (1931), and Gerard and Hyman (1931) found that *Paramecium caudatum* was resistant to cyanide. However, Child (1941) refers to unpublished data obtained by Hyman, in which she found a considerable decrease in O_2 consumption of *Paramecium* in KCN. Dr. Hyman[2] has also informed the author by personal communication that she

[1] These investigations were partly supported by a grant-in-aid received from the Society of Sigma Xi. The Barcroft-Warburg apparatus was purchased by a grant furnished by Mr. Arthur S. Raymond of the Lincoln Drug Co., Lincoln, Nebraska.

[2] Dr. Libbie H. Hyman has granted me the privilege of using the following communication which she sent to me at my request: "Some years ago, being skeptical of Lund's failure to find any cyanide-sensitive respiration in Paramecium, I spent a great deal of time and effort in testing the action of cyanide on the oxygen consumption of Paramecium, using Winkler's method. I met with so many difficulties that I never published the results; chief among them were the impossibility of measuring equal suspensions of Paramecium from a volumetric pipette because the animals adhere to the glass, and the toxicity to Paramecium of all waters except the culture water, which in itself has high oxygen consuming powers. However, my results indicated that starved Paramecium have no cyanide-sensitive respiration, in agreement with the finding of Lund, but non-starved ones have about 35 per cent such respiration. After giving up the work as impractical by my methods, I sought the help of Dr. Gerard. Dr. Gerard kindly consented to test the matter on his manometers but failed to find any depressing action of cyanide on non-starved Paramecium. As I played no role in this work except that I furnished the Paramecium, I feel that Dr. Gerard was over-generous in making me co-author. I was not satisfied with these results, first, because successive manometric readings were highly variable, and second, because the buffer solution used was toxic to Paramecium, depressing oxygen consumption by about 50 per cent in itself.

"As a cyanide sensitivity of the extra oxygen consumption caused by feeding was indicated in my experiments, it became interesting to know the nature of this extra respiration. I therefore attempted to compare the effects on oxygen consumption of the ingestion by Paramecium of particles without food value (carbon suspension) and of particles with food value (yeast). Here, again, I met with insuperable difficulties. I could never get any sample of yeast, no

found an inhibition of O_2 consumption in *P. caudatum* when it was exposed to HCN.

Sato and Tamiya (1937) claimed that they found cytochrome a and c in *Paramecium*. If this is true, then it is difficult to understand the insensitivity of the respiratory mechanism of this species to HCN. Because of these observations and of the unpublished results of Hyman, and since studies have not been made on the sensitivity of Paramecium to cyanide when proper KOH–KCN mixtures are used as absorption media (Krebs, 1935), the following investigation was carried out.

Material and Methods

Two species were used in this work, *Paramecium caudatum* and *Paramecium aurelia*. The culture solution used was highly buffered and was the same as was used later in the flasks of the Barcroft-Warburg apparatus for testing. The solution consisted of $K_2HPO_4.H_2O$ — 80 mg., KH_2PO_4 — 80 mg., $CaCl_2$ — 104 mg., Mg_3PO_4 — 2 mg., and redistilled water to make one liter.

In making up the stock culture, 15 gms. of timothy hay were boiled in 500 ml. of this solution for one-half hour, after which the solution was made up to its original volume by the addition of distilled water. This "broth" was then diluted further by the addition of the above buffered solution to make 4000 ml. The hydrogen ion concentration was held at pH 7.0 $\pm$ 0.2.

This culture solution, along with approximately 3 gms. of sterile hay, was put into chemical bottles with 500 ml. capacity and moderately narrow necks (3–4 cm. in diameter). About 4000 paramecia were added to each container. Within 5 days they became extremely numerous, especially in the neck region of the bottle whence they could be removed easily in large numbers.

The Barcroft-Warburg apparatus was used for ascertaining rate of oxygen consumption. The shaking mechanism was adjusted to operate at 110 complete cycles per minute. Because of the possibility of NH_3 production (Specht, 1934), a 0.3 ml. portion of 0.3 N HCl was added to the side arm (onset) of each manometer flask.

During the course of these investigations, various test solutions were made up containing different concentrations of KCN as follows: 0, 10^{-5}, 10^{-4}, and 10^{-3} M. Corresponding KOH–KCN absorption solutions were made up for each concentration of test solution according to Krebs (1935), and 0.4 ml. of the proper mixture (Pace and Belda, 1944) was added to the inner well (inset) of each flask containing organisms in KCN. To the inset of each of the flasks in which the test solution contained no KCN, a 0.4 ml. portion of M KOH was added.

A typical test was made in the following manner: Paramecia were drawn off from the top of the bottles in which they were cultured and placed in 15 ml. centrifuge tubes in which they were washed several times in fresh solution by careful centrifugation. The only time the organisms were subjected to centrifugation was

matter how many times boiled and centrifuged, that did not have high oxygen consuming powers, and all carbon suspensions also remove oxygen from the medium. However, there were indications that ingestion of a non-nutritive substance can cause as great an increase in oxygen consumption as does ingestion of food. This suggests that the extra respiration of feeding does not result from an oxidation of the food material."

during the washing process and this was carried out with great care by means of a hand centrifuge. An attempt was made to have between 2000 and 3000 *P. aurelia* or 1000 and 2000 *P. caudatum* in each 5 ml. sample. A count was always made of the organisms in each flask at the end of an experiment.

In all the tests reported here, those organisms designated "young" paramecia were taken from 5–7 day-old cultures; those designated "old" paramecia, from 15–20 day-old cultures; those designated "starved" paramecia were "old" organisms that had been placed in inorganic buffer solution without food material for 2 or 3 days. The "young" paramecia had much more food material present in the form of food vacuoles than the "old" paramecia.

Results

Effect of cyanide on respiration in Paramecium aurelia

Paramecium aurelia was the first species studied. It is a much smaller form than *P. caudatum*, but its rate of respiration per unit volume is similar to the latter (Pace and Kimura, 1944).

A number of tests were made at various KCN concentrations. Organisms that were taken from cultures 15–17 days after they had been started (i.e., "old" paramecia) were used in most of the tests. They were washed by centrifugation in the solution given above, and then divided into two portions. KCN was added to one of these portions in the concentrations designated in the table. Several tests were also carried out on starved paramecia and young paramecia. The results are presented in Table I.

P. aurelia was found to be sensitive to KCN in all the tests made, except where starved individuals were used. The normal average oxygen consumption for organisms taken from the 15 or 17 day-old cultures was 6.31 mm^3 per hour per mm^3 of cell substance at 25° C. This compares favorably with the results of Pace and Kimura (1944) who found that *P. aurelia* consumed oxygen at the rate of 6.16 mm^3 per hour per mm^3 of cell substance at 25° C.

The presence of food material may have something to do with the fact that in all the tests made, the younger paramecia showed a much greater sensitivity to cyanide than the older. In fact, starved specimens were insensitive to cyanide. When exposed to KCN at a concentration of 10^{-4} M, respiration in the young organisms was inhibited on the average by about 40 per cent. The respiration of old organisms showed an average inhibition of 22 per cent to the same concentration of KCN. At KCN concentrations of 10^{-3} M, inhibition of respiration was greater than with the lower concentration, but the results were similar insofar as young and old organisms are concerned. In young paramecia, the average O_2 consumption (1318 mm^3 O_2 per hour per million) in the buffered solution without KCN was about twice that in old organisms. An average O_2 consumption of 640 mm^3 was found for the young paramecia when they were exposed to 10^{-3} M KCN. Thus the cyanide at this concentration results in a 50 per cent inhibition in respiration in *P. aurelia*.

Effect of KCN on respiration in Paramecium caudatum

Paramecium caudatum has been studied to a much greater extent than *P. aurelia* and, as brought out previously, all the work (except for unpublished early

TABLE I

The effect of KCN on respiration in *Paramecium aurelia*. *, starved specimens; 5–7 day cultures, young specimens; all others, old specimens. Temperature, 25° C.; pH, 7.0 ± 0.2. Average volume of one million paramecia, 121.4 mm.³ (this does not include the volume of starved specimens). Each figure in columns 4 and 5 represents the average for 3 tests.

Molar concentration of KCN	Age of culture in days	Duration of test in hours	Average O_2 consumption in mm.³ per hour per million	Average O_2 consumption in mm.³ per hour per mm.³ of cell substance	Per cent inhibition
0	17*	4	462		None
10^{-4}			484		
0	16	3	746	6.14	19.9
10^{-4}			598	4.92	
0	16	3	680	5.60	29
10^{-4}			485	3.99	
0	15	5	709	5.84	36.1
10^{-4}			453	3.73	
0	15	3	808	6.65	18
10^{-4}			665	5.47	
0	15	3	841	6.92	12
10^{-4}			747	6.15	
0	7	3	906	7.46	28.5
10^{-4}			657	5.42	
0	5	5	1360	11.20	42
10^{-4}			788	6.49	
0	16*	3	520		None
10^{-3}			511		
0	15	3	818	6.73	32
10^{-3}			557	4.58	
0	5	3	1516	12.48	60
10^{-3}			605	4.98	
0	6	5	1120	9.22	40
10^{-3}			677	5.57	

results of Dr. Libbie H. Hyman) indicates that *P. caudatum* is insensitive to cyanide. One great difference in the work reported here and previous investigations carried out on the effect of cyanide on *Paramecium* is that in these experiments suitable KCN–KOH absorption mixtures rather than pure KOH were used in the manometer flasks to prevent absorption of HCN from the test solution.

The same procedures were followed here as for *P. aurelia*. The results are presented in Table II.

As indicated by the results, much variation was found in the action of KCN on *Paramecium caudatum*. In the first few tests very great difficulty was experi-

TABLE II

The effect of KCN on oxygen consumption in *Paramecium caudatum*. *, starved specimens; 5 day cultures, young specimens; all others, old specimens. Temperature, 25° C.; pH, 7.0 ± 0.2. Average volume of one million paramecia, 591 mm.³ Each figure in columns 4 and 5 represents the average for 3 tests.

Molar concentration of KCN	Age of culture in days	Duration of test in hours	Average O_2 consumption in mm.³ per hour per million	Average O_2 consumption in mm.³ per hour per mm.³ of cell substance	Per cent inhibition
0	16*	3	1565		None
10^{-5}			1518		
0	16	2	3273	5.53	15.5
10^{-5}			2734	4.62	
0	16	6	3734	6.33	15
10^{-5}			3181	5.37	
0	5	9	4420	7.47	30
10^{-5}			2650	4.48	
0	17	3	3040	5.14	None
10^{-4}			3010	5.09	
0	19	5	2700	4.56	27
10^{-4}			1978	3.34	
0	15	3	3787	6.40	40
10^{-4}			2243	3.80	
0	5	4	4270	7.22	42
10^{-4}			2475	4.18	
0	16*	5	1190		None
10^{-3}			1280		
0	15	3	3580	6.05	42
10^{-3}			2072	3.50	
0	5	12	4590	7.76	66
10^{-3}			1560	2.63	
0	15	4	4170	7.05	43
10^{-2}			2380	4.02	

enced, chiefly because some apparently minor details in manipulation were overlooked and this may have had a very noticeable effect on the results. It was suspected from the results of the first few tests that food played an important part in the degree of sensitivity of these organisms to KCN. For this reason several tests were conducted on this species under the same type of conditions as was used for *P. aurelia*, namely: (1) young paramecia (5 day cultures), (2) old paramecia (15 to 19 day cultures) and (3) starved paramecia.

The results indicate that although there was great variation in some of them, the young specimens show a greater sensitivity to KCN. The starved specimens proved to be non-sensitive. In some tests there appeared to be an actual accelera-

tion of O_2 consumption when starved *P. caudatum* was put into KCN solutions but the results may have been due to experimental error. They are not included in the table. In one test (included in table) which was made upon old organisms, there was no evidence of cyanide sensitivity; no explanation can be given for this exception.

The average inhibition of O_2 consumption found in old *P. caudatum* exposed to solutions containing 10^{-5} M KCN was approximately 15 per cent; in solutions containing 10^{-4} M, 33 per cent; and in solutions containing 10^{-3} M, 42 per cent. In young *P. caudatum* exposed to 10^{-5} M KCN, respiratory inhibition was approximately 30 per cent; in solutions containing 10^{-4} M KCN, 42 per cent; and in solutions containing 10^{-3} M, approximately 66 per cent. Thus, inhibition of oxidative metabolism increases with increase in KCN concentration, and the degree of sensitivity to cyanide seems to depend upon the quantity of food material present. This is in agreement with the results of Hyman. Higher concentrations than 10^{-3} M KCN were attempted but the results are meaningless because of such extreme variations and for this reason they have not been included in this report.

Effect of dextrose on the degree of inhibition by cyanide

Many workers have reported that one of the factors in the sensitivity of the respiratory mechanism to cyanide is the degree of carbohydrate saturation in the cell. Keilin (1932) suggests that perhaps the most important factor concerned with cellular sensitivity to cyanide is the concentration of carbohydrate. Commoner (1939) working with bakers' yeast, Emerson (1927) with *Chlorella,* and Hall (1941) with *Colpidium campylum,* all found either a greater inhibition with cyanide when dextrose was present or no inhibition without dextrose.

Since it is highly probable that a large portion of the food material of *Paramecium* is carbohydrate and since it was found that the greatest sensitivity to cyanide occurred when the greatest quantity of food was present, it was thought advisable to run respiration tests with the organisms in a dextrose solution.

Old paramecia were selected and washed in the buffered test solution containing 0.01 M dextrose. Then the solution containing the paramecia was divided

TABLE III

The effect of KCN on *Paramecium caudatum* in a 0.01M dextrose-buffer solution. All the organisms were taken from 16 to 19 day-old stock cultures. Temperature, 25° C.; pH, 7.0 ± 0.2. Average volume of one million paramecia, 580 mm.³ Each figure represents the average for 3 tests.

Molar concentration of KCN	Age of culture in days	Duration of test in hours	Average O_2 consumption in mm.³ per hour per million	Average O_2 consumption in mm.³ per hour per mm.³ of cell substance	Per cent inhibition
0	16	4	4550	7.84	48
10^{-4}			2360	4.06	
0	16	5	3860	6.65	51
10^{-4}			1890	3.25	
0	19	3	4120	7.10	54
10^{-4}			1895	3.26	

into two portions. To one portion, KCN was added to 10^{-4} M; the other portion was used as control. This experiment was repeated twice and the results are presented in Table III.

The results show that the rate of respiration in *Paramecium caudatum* is increased with the addition of dextrose to the test solution. The average rate of respiration in the dextrose-buffer solution for all tests without KCN added was 4170 mm^3 per hour per million organisms as compared to an average 3470 mm^3 in the same type of organisms tested in the buffer solution without dextrose (Table II). They also show that there was an average inhibition of 51 per cent in O_2 consumption in 10^{-4} M KCN in the dextrose-buffer solution which is much greater than the average inhibition in 10^{-4} KCN without dextrose. The average inhibition for two experiments in which the latter solution was used, was 33.5 per cent; in one of the experiments there was no inhibition whatever, but this has not been included in the average.

Discussion

Many factors may have contributed to the failure of earlier investigators to find inhibition in respiration in *Paramecium* when exposed to cyanide. Considerable error must have been caused by the absorption of free HCN by the KOH used as absorption fluid. The initial inhibitory effect followed by an increase in oxygen consumption noted in the results of Pitts (1932) and Lwoff (1934) is evidently due to the fact that little attention was given to the rapid absorption of cyanide (via distillation of HCN) by the absorption fluid. Hall (1941), using suitable KOH–KCN mixtures as absorption media, proved conclusively that respiration in *Colpidium* was cyanide sensitive.

In the investigations reported here, care was taken to prevent distillation of HCN over into the absorption fluid. However, there is another factor that may or may not have been realized by these earlier workers, namely, the food content of the paramecia with which they worked. It is possible that the organisms used by them were taken from "old" cultures and hence had comparatively little food material in them. If this be true, it explains their failure to find inhibition in respiration, for, as reported above, sensitivity seems to depend, at least partly, upon the food content of *Paramecium*. This very important factor was noted some twenty years ago by Dr. Libbie Hyman (see footnote 2).

In these experiments, the organisms were taken from the culture solution, washed, and placed in fresh test solution, and then put into manometer flasks, all within 10–15 minutes. Thus in most of the tests the organisms were actually in inorganic solution without food for 3.5 hours; in some tests 4.5–5.5 hours, but rarely longer than this. During this time, very little change could be noted in food vacuole content or size. It was also noted that respiration varied very little, if at all, from the beginning to the end of a test. In other words, the decrease in food content is so slight within this short period of time that there was no noticeable change in rate of respiration.

Carbohydrate makes up a great portion of the food of *Paramecium*. One of the most important factors in the degree of sensitivity of respiration to KCN, etc. is the concentration of carbohydrate in the cell. Thus when dextrose was added to the buffer solution in which the respiration of *Paramecium caudatum* was tested, the per cent inhibition was greater than in the buffer solution without dextrose.

Summary

1. The oxygen consumption in *Paramecium caudatum* and *Paramecium aurelia* is partially inhibited by potassium cyanide.

2. The extent of inhibition by cyanide is dependent upon the food content of the organisms as well as upon the concentration of cyanide in the solution.

3. In *P. aurelia,* starved specimens are insensitive to cyanide; old specimens are not as sensitive as young. In 10^{-4} M KCN respiration in the old organisms was inhibited by approximately 22 per cent while in the young organisms it was inhibited by approximately 40 per cent.

4. In *Paramecium caudatum,* starved specimens were non-sensitive to KCN; old specimens exposed to 10^{-3}, 10^{-4}, and 10^{-5} M KCN show, respectively, a 42, 33, and 15 per cent inhibition in respiration. Young specimens, exposed to 10^{-3}, 10^{-4}, and 10^{-5} M KCN show, respectively, a 66, 42, and 30 per cent inhibition.

5. The inhibition in the rate of respiration in *P. caudatum* was greater in buffer solution plus dextrose (0.01 M) than in the same solution without dextrose.

6. The effect of cyanide on respiration in *Paramecium* depends upon the degree of saturation of the respiratory mechanism with carbohydrate.

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THE AGGLUTINATION OF STARFISH SPERM BY FERTILIZIN[1]

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Agglutination of starfish sperm by specific egg water (supernatant sea water from egg suspensions) has never been clearly demonstrated. Glaser (1914) and Woodward (1918) reported a strong agglutination of *Asterias forbesii* sperm by homologous egg water, but Just (1930) was unable to confirm this work. Attempts to demonstrate agglutination of sperm by egg water in other species of starfish have failed. Thus Loeb (1914) observed no reaction in *Asterias* (probably *Pisaster*) *ochraceus,* and Tyler (1941) had a similar result with *Patiria miniata.* From this it might appear that fertilizin is not present in starfish egg water. However, Tyler found that treatment of *Patiria* sperm with egg water lowered the fertilizing power of the sperm. Tyler (1941, 1942) interpreted this as support for his view that fertilizin may exist naturally in a non-agglutinating "univalent" form. An individual molecule of such univalent fertilizin should have but one combining group capable of reacting with groups (antifertilizin) on the sperm surface. On the basis of the Marrack-Heidelberger (1938) lattice theory, univalent fertilizin should therefore combine with but not agglutinate these cells. Tyler suggests that such univalent fertilizin may be present quite generally in forms showing no agglutination of sperm by egg water. He therefore supports the belief held by Lillie (1919) and Just (1930) that fertilizin occurs universally.

In view of the concept of univalent fertilizin and the provisional status of the starfish with respect to sperm agglutination by egg water, it is of some interest that sperm of certain starfish agglutinate when mixed with homologous egg water and an "adjuvant." The first adjuvant found was lobster (*Panulirus*) serum. The agglutination reaction was discovered accidentally in the course of studies on the natural agglutinins in lobster serum (Tyler and Metz, 1944). In an attempt to separate natural agglutinins for *Patiria* eggs and sperm, the serum was treated with eggs and then titrated for sperm agglutinins. The treatment with eggs increased the sperm agglutinin titer several fold. Investigation of this unexpected result showed that sperm absorbed lobster serum (freed of natural sperm agglutinins), when mixed with *Patiria* egg water, agglutinated *Patiria* sperm. Tests on other material showed the presence of adjuvant in hen's egg white. A preliminary report (Metz, 1944) on this work has already appeared. The studies confirm Tyler's view that fertilizin is present in *Patiria* egg water. However, the experiments indicate that this fertilizin is multivalent. Data are given which suggest that normal *Patiria* sperm is "univalent" with respect to exposed antifertilizin groups, but that more of these groups are "exposed" by the adjuvant.

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Material and Methods

The Pacific webbed star, *Patiria miniata*, was used as standard material. The Pacific star *Pisaster ochraceus*, the Pacific sand star *Astropecten* sp. and the Atlantic *Asterias forbesii* were used in confirmatory and specificity tests.

Egg and sperm suspensions were prepared from ripe extirpated gonads. These organs were minced in a measured volume of sea water and then filtered through bolting cloth to remove the gonadal tissue. The difference in volume of the filtrate and the sea water initially added gives the volume of "dry" (undiluted) material. Egg and sperm dilutions were reckoned from this "dry" volume. Egg water solutions were obtained by drawing off the supernatant from standing egg suspensions (25–50 per cent of dry eggs in sea water), or by heating such suspensions and filtering or centrifuging off the eggs.

Lobster (*Panulirus interruptus*) serum was obtained by drawing blood from the heart and allowing it to clot. After syneresis the serum was drawn off. Since *Panulirus* serum contains natural heteroagglutinins for sperm of various organisms (Tyler and Metz, 1944) including *Patiria*, *Pisaster* and *Astropecten*, it is impractical to use the untreated serum. By absorption with *Patiria* sperm the natural agglutinins for *Pisaster* and *Astropecten* as well as *Patiria* sperm can be removed. Such absorbed serum was used as the adjuvant for sperm of all three species. For reasons of economy both in material and time, hen's egg white was used as the adjuvant in the later experiments. This material was made isotonic by adding one volume of concentrated (1.73 ×) sea water. It was then diluted to 20 per cent with normal sea water and filtered to remove the mucin, chalazae and other insoluble material. This diluted egg white was usually heated to 100° C. and filtered or centrifuged since this procedure increased its activity several fold. Hen's egg white does not contain natural agglutinins for *Patiria*, *Pisaster* or *Astropecten* sperm. Thus, initial absorption with starfish sperm was not necessary.

Assays of unknown egg water were made by diluting the unknown solution in twofold steps with sea water and then adding constant amounts of adjuvant-treated sperm to each dilution of unknown egg water. Adjuvant was titrated in a similar manner. However, when titrating adjuvant, constant amounts of sperm suspension were added to the dilutions of unknown adjuvant. Subsequently, constant amounts of egg water were added to each adjuvant dilution. Less satisfactory results are obtained if any other order of mixing is employed in this test. In all cases the presence or absence of agglutination was determined by microscopical examination one to several minutes after mixing. Titers were recorded as the highest dilution of unknown showing agglutination of the test sperm.

The apparatus and methods used in ultraviolet irradiation have been described in a previous article (Metz, 1942).

Activation and Agglutination of Starfish Sperm

In these studies no definite agglutination of *Patiria*, *Pisaster*, *Astropecten* or *Asterias* sperm was observed following the addition of homologous egg water. However, *Patiria* sperm suspensions frequently appeared "granular" after this treatment. These microscopic "granules" consisted of two or three sperm fixed together and represent a plus-minus agglutination reaction. Various devices such

as centrifugation were employed in an attempt to bring this reaction to a distinct agglutination, but all of them failed.

The starfish sperm in dilute (0.5 to 1.0 per cent) sea water suspension were virtually immobile. The cells did not respond to treatment with fresh sea water (lowering CO_2 tension) or with homologous egg water. Starfish sperm thus differ from *Arbacia* and *Nereis* sperm which become more active when diluted with sea water (Lillie, 1913; Just, 1930), and from *Arbacia* (Lillie, 1913), *Strongylocentrotus* (Tyler, 1939) and *Megathura* sperm (Tyler, 1940) which become intensely motile when mixed with homologous egg water.

Patiria, Pisaster and *Astropecten* sperm, although refractory to treatment with sea water and egg water, nevertheless became intensely active when treated with isotonic hen's egg white or the serum of the lobster (*Panulirus*), fish (*Crassius*), hen, or rabbit. Furthermore, adjuvant-treated sperm of these starfish agglutinated strongly on addition of homologous egg water. *Asterias* was tested on three successive seasons. The sperm became intensely active when treated with isotonic hen's egg white. Weak agglutination sometimes occurred after addition of homologous egg water to the sperm egg white suspension. Unfortunately, the agglutination was so weak and occurred so irregularly that quantitative studies could not be made.

In *Patiria* the agglutination resulting from treatment of sperm with adjuvant and egg water was exclusively head to head. Each clump consisted of a central mass of sperm heads tightly bound together, and a peripheral region of free tails which projected out radially from the central mass of heads. *Patiria* thus differs from *Megathura,* since sperm of the latter agglutinate tail to tail as well as head to head (Tyler, 1940). The clumped *Patiria* sperm soon became immobile even though the free sperm remained active for an hour or more. The spontaneous reversal of agglutination so characteristic of the sea urchin occurred to a limited extent only after the free sperm had become inactive.

Properties of Patiria Fertilizin

Fertilizin may be defined by the following properties: (1) it combines with (but does not necessarily agglutinate) sperm, (2) it is highly specific in this reaction, and (3) it is obtained primarily from eggs. Studies on the role of egg water in the agglutination of treated sperm show that *Patiria* egg water has these properties.

Absorption of Patiria egg water by sperm. A direct combination between sea urchin fertilizin and sperm may be demonstrated by absorption of egg water with sperm, or by neutralization of egg water with appropriate sperm extract (Lillie, 1913; Frank, 1939). Similarly, it may be shown that sperm-absorbed *Patiria* egg water will no longer agglutinate treated sperm. Indeed, complete exhaustion of the egg water may be attained even in the absence of adjuvant. In a typical experiment 20 drops of *Patiria* egg water were mixed with 22 drops of concentrated (25–50 per cent) *Patiria* sperm. The mixture was set aside to allow for reaction. Twenty drops of the same egg water were mixed with 22 drops of sea water to serve as a control. After centrifugation the fluid of both tubes was titrated with adjuvant-treated (0.5–1 per cent) sperm. The undiluted absorbed egg water did not agglutinate the sperm, whereas the control unabsorbed egg water clumped the sperm

even at a dilution of $\frac{1}{256}$ of full strength. Other controls showed that sperm without adjuvant were not agglutinated by control or absorbed egg water, or by the adjuvant (*Patiria* sperm-absorbed *Panulirus* serum). Thus a substance (fertilizin) is present in *Patiria* egg water which will combine with specific sperm independently of the adjuvant. The adjuvant is required only for agglutination.

Specificity of starfish fertilizin. The reaction between sperm and fertilizin is characterized by a high order of specificity (Tyler, 1940). Cross tests between *Patiria, Pisaster* and *Astropecten* sperm and fertilizin show that these starfish are not exceptional in this respect. Sperm suspensions of the three species were treated with *Patiria* sperm-absorbed *Panulirus* serum, and then cross tested with the egg waters of the three species. The data are given in Table I.

TABLE I

Specificity of Patiria, Pisaster *and* Astropecten *egg waters*

		Patiria sperm	*Pisaster* sperm	*Astropecten* sperm
Patiria egg + water	adjuvant	+++	±	−
	sea water	±	−	−
Pisaster egg + water	adjuvant	−	+++	−
	sea water	−	++	−
Astropecten egg + water	adjuvant	−	−	±
	sea water	−	−	−
	adjuvant + sea water	−	−	−
	Patiria sperm supernatant	−	−	−

It will be seen that *Patiria* and *Pisaster* egg waters agglutinated only homologous sperm. Thus the species specificity rule holds for these two forms. In this experiment *Pisaster* egg water clumped homologous untreated sperm. This reaction did not occur with predictable regularity. The reaction between *Astropecten* egg water and homologous sperm was doubtful. This may be ascribed to neutralization of the *Astropecten* egg water by the *Patiria* sperm supernatant present in the adjuvant solution. The relationship here is somewhat involved. With the exception of the reaction between *Patiria* sperm supernatant and *Astropecten* egg water, the reactions were species specific.

The source of Patiria fertilizin. Only egg water prepared from suspensions of *Patiria* eggs possessing their normal gelatinous coats agglutinated species sperm in the presence of the adjuvant. Blood from female animals did not have this effect. Thus it may be concluded that a specific substance is obtained from starfish eggs which will react with and under certain conditions agglutinate species

sperm. This then gives clear and direct support to Tyler's (1941) view that fertilizin exists in the *Patiria* egg water.

The Nature of Patiria Fertilizin

Tyler (1941) concluded that *Patiria* fertilizin was univalent for combining groups complementary to sperm. It follows from the lattice theory that such univalent fertilizin must become multivalent to agglutinate the sperm. The adjuvant should then convert the natural univalent *Patiria* fertilizin to a multivalent, agglutinating form. However, another possibility in accord with the lattice theory is that the fertilizin is multivalent but the sperm is normally univalent. The results of the following experiments favor this latter view.

Effect of ultraviolet light on Patiria fertilizin. Sea urchin fertilizin can be converted to the univalent form by proper exposure to heat, enzymes, x-radiation and ultraviolet light (Tyler, 1941; Metz, 1942). Such treated fertilizin will not agglutinate sperm but it will combine with sperm rendering the sperm unagglutinable by untreated fertilizin. To test for the possibility of a similar action, *Patiria* fertilizin was exposed to ultraviolet irradiation. It was found that irradiated *Patiria* fertilizin will not agglutinate adjuvant-treated homologous sperm, and normal fertilizin will not subsequently agglutinate the sperm that has been treated with irradiated fertilizin. Thus it is possible that the natural fertilizin is multivalent and the irradiated material is true univalent fertilizin. The data from a typical experiment are given in Table II.

Table II

Destruction of agglutinating power of Patiria *fertilizin by ultraviolet light and agglutination inhibiting properties of this fertilizin*

Solution	Irradiated fertilizin	Control fertilizin	Irradiated fertilizin + control fertilizin	Irradiated sea water + control fertilizin
Reaction of adjuvant-treated sperm	−	++++	−	++++

Two stender dishes each containing 5 cc. of a *Patiria* fertilizin solution and one dish containing 5 cc. of sea water were irradiated for 220 minutes. The control fertilizin sample was screened from the ultraviolet light by a "noviol C" filter. After the irradiation the control and irradiated fertilizin samples were tested for agglutinin activity by mixing 2 drops of hen's egg white treated sperm (1%) with 2 drops of each fertilizin solution. At the same time 2 drops each of the sperm and irradiated sea water were mixed. It will be seen that the irradiated fertilizin was inactive whereas the control strongly agglutinated the sperm. After this examination one drop of unirradiated test fertilizin was added to the irradiated fertilizin-adjuvant-sperm mixture and one drop to the irradiated sea water-adjuvant sperm. In this test for inhibition of agglutination it will be seen that sperm treated

with irradiated fertilizin did not agglutinate upon subsequent addition of normal fertilizin, whereas the sperm treated with irradiated sea water reacted strongly.

Agglutination of adjuvant-free Patiria sperm by fertilizin. More definite evidence for the multivalent nature of starfish fertilizin was obtained from a study of the effect of adjuvant on sperm. Adjuvant was added to *Patiria* sperm and then removed. Such adjuvant free sperm agglutinated on addition of natural fertilizin. Twenty drops of 0.5 per cent sperm were mixed with 10 drops of isotonic hen's egg white. A control sample consisted of 20 drops of 0.5 per cent sperm plus 10 drops of sea water. Both samples were centrifuged and the packed sperm was resuspended in 20 drops of sea water. Two drops of sperm from each were tested with *Patiria* fertilizin. The control suspension did not react whereas the sperm centrifuged from the egg white agglutinated moderately. The suspensions were recentrifuged and the supernatants tested and found free of adjuvant. The sperm masses were resuspended in 16 drops of sea water after the second centrifugation and tested. The control sperm did not react to fertilizin whereas the sperm previously treated with adjuvant agglutinated weakly.

This experiment was not confirmed with *Astropecten*. *Astropecten* sperm after centrifugation from hen's egg white solution were not agglutinated by homologous fertilizin alone, although this sperm reacted strongly when both egg white and fertilizin were added.

It seems clear then that *Patiria* fertilizin will agglutinate sperm after the adjuvant has been removed from the sperm. It may therefore be concluded that the natural *Patiria* fertilizin is multivalent.

Univalent Sperm

Evidence has just been presented to show that natural *Patiria* fertilizin is multivalent and capable of agglutinating sperm. It follows that the normal sperm is incapable of agglutination. The adjuvant must then convert the sperm to an agglutinating condition.

It seems unlikely that stimulation of the normally immobile sperm to intense activity is of any considerable importance in this adjuvant-fertilizin agglutination of *Patiria* sperm since immunological doctrine does not require motility of cells for agglutination. Thus non-motile bacteria and erythrocytes agglutinate strongly when mixed with specific antibody. Furthermore, heat killed sea urchin sperm agglutinate strongly on addition of fertilizin. However, heat killed *Patiria* sperm did not react when mixed with fertilizin and adjuvant. The deficiency of the normal sperm must then involve the antigenic structure of the cell surface. For agglutination to occur the area of the sperm surface containing groupings complementary to fertilizin must be rather extensive. If this region of the sperm surface were limited in extent and contained but a few or even a single antifertilizin group, the sperm could be considered "univalent" for this particular antigen. Such sperm should not agglutinate when mixed with complementary agglutinin (fertilizin). At best only two or three sperm could clump together. This condition is occasionally observed when untreated *Patiria* sperm and fertilizin are mixed. It has been described as the "granular" reaction.

Absorption of Patiria fertilizin by treated and normal sperm. If normal *Patiria* sperm are "univalent" with respect to exposed antifertilizin groups, the cells must

be made multivalent before they can be expected to agglutinate. The adjuvant is believed to effect such a conversion to the multivalent form by "exposing" latent or unreactive antifertilizin present on or near the sperm surface. Treated sperm then should bind more fertilizin than the normal "univalent" sperm. One of three absorption experiments demonstrating this is recorded in Table III.

TABLE III

Absorption of fertilizin by sea water and egg white treated Patiria *sperm*

Absorbing Mixtures			
Tube I	Tube II	Tube III	Tube IV
0.5 cc. sea water 0.5 cc. fertilizin 0.5 cc. sperm	0.5 cc. egg white 0.5 cc. fertilizin 0.5 cc. sperm	0.5 cc. egg white 0.5 cc. fertilizin 0.5 cc. sea water	0.5 cc. sea water 0.5 cc. fertilizin 0.5 cc. sea water

Titration of absorbed fertilizin solutions

Dilution of absorption supernatant	Tube I	Tube II	Tube III	Tube IV
1/2	+++	−	++++	++++
1/4	++	−	++++	++++
1/8	+	−	++++	++++
1/16	+	−	+++	+++
1/32	+	−	+++	+++
1/64	+	−	++	++
1/128	+	−	++	++
1/256	−	−	++	++
1/512	−	−	+	+
1/1024	−	−	+	+
1/2048	−	−	−	−

Four absorption tubes were prepared as indicated in the table. Fifty per cent sperm was used in the absorption and raw isotonic hen's egg white was employed as adjuvant. The tubes were refrigerated for nine hours to allow for complete reaction, and then centrifuged. The supernatants were then titrated for fertilizin with one per cent treated sperm. In absorption tubes III and IV sea water was substituted for the sperm added to tubes I and II. No adjustment was made in the titration for the volume of absorbing sperm removed from I and II by centrifugation. This is justified since the titration was made on a comparative basis and tubes III and IV represent controls for neutralization of fertilizin by egg white. Furthermore, the error in absolute values introduced by this involves something less than $\frac{1}{6}$ of a dilution and therefore is well within the error of the method. Likewise, no adjustment was made in the supernatant of tube I for the adjuvant present in the absorption supernatant of tube II. Such adjustment was apparently unnecessary since the titers of the control tubes III and IV were the same (1024) The titers of these tubes also show that the egg white does not neutralize fertilizin. Comparison of tubes I and III shows that the sea water-sperm mixture caused an 8- to 64-fold drop in fertilizin concentration. However, in tube II (titer 0)

the adjuvant-sperm mixture completely exhausted the fertilizin. The striking difference in the titers of tubes I and II (128 and 0 respectively) demonstrates clearly that treated sperm has a greater fertilizin binding capacity than normal sperm.

Effect of the Adjuvant on the Fertilizing Power of Patiria Sperm

Since the adjuvant increases the fertilizin binding power of sperm and also the motility of these cells, it seemed likely that treated sperm would be unusually effective in fertilization. Several experiments comparing the treated and normal sperm in this respect showed this to be the case. The results of one such experiment are given in Table IV.

TABLE IV

The effect of hen's egg white on the fertilizing power of Patiria *sperm*

Sperm dilution	Egg white treated sperm	Sea water treated sperm	Egg white + Patiria eggs	Sea water + Patiria eggs
	% cleavage	% cleavage	% cleavage	% cleavage
1/2	94% (75)*	38% (95)*	0.7% (152)*	0.0% (118)*
1/4	95% (66)	0% (58)		
1/8	89% (45)	7.4% (54)		
1/16	88% (50)	6.8% (74)		
1/32	89% (53)	2.0% (65)		

* Total number of eggs counted.

A fresh one per cent sperm suspension was divided into two parts. One part was diluted serially (in twofold steps) with boiled isotonic hen's egg white. The other part was diluted similarly but with sea water. Sperm dilutions are given as the dilution of one per cent sperm added to the eggs. One drop of each sperm suspension was added to twelve drops of *Patiria* eggs in 6 cc. of sea water. To control for parthenogenesis one drop of egg white was added to one dish of eggs and a drop of sea water to a second dish. The eggs were examined for cleavage three hours after addition of sperm.

Although the number of eggs counted was small it can readily be seen that the egg white treatment greatly increased the fertilizing power of the sperm. Even at the lowest dilutions the treated sperm was twice as effective as the untreated cells. At high dilutions the treated sperm fertilized nearly 90 per cent of the eggs whereas the normal sperm fertilized less than 10 per cent. Gray (1915) has reported a similar result with alkali treated *Asterias glacialis* sperm.

Specificity of the Adjuvant

Although no exhaustive search was made for different sources of adjuvant, a number of unrelated preparations were encountered which stimulated *Patiria* sperm and rendered it agglutinable by fertilizin. These preparations included *Panulirus*, rabbit, fish (*Crassius*), and hen sera, and hen's egg white. Thus the source of the adjuvant is not highly specific.

Properties of the Egg White Adjuvant

The adjuvant action can not be attributed to the high pH of raw egg white (Needham, 1931) since the material is active at sea water pH. Therefore, preliminary attempts were made to characterize an "active principle" in the hen's egg white. The agent is quite heat stable. Its activity was retained even after several hours at 100° C. In fact heating increased the activity of the egg white several fold. Ultraviolet light had a similar effect. This suggests the release of inactive bound agent. The "active principle" was quite nondialyzable both before and after heating. It was soluble in saturated ammonium sulfate, but insoluble in strong acetone and alcohol. Thus it is probably neither ordinary protein nor lipoid.

Discussion

From the evidence presented it is concluded that fertilizin is obtained from *Patiria* eggs, and that this fertilizin, although it does not agglutinate normal sperm, is a multivalent agglutinin that reacts with the normal sperm. It is further believed that the exposed antifertilizin of normal *Patiria* sperm is limited to a small area of the sperm surface and contains only a few or even a single combining group complementary to fertilizin. For practical purposes such sperm may be considered "univalent." It is necessary to assume that some antifertilizin is exposed on the normal sperm to explain the absorption of fertilizin by such sperm and to account for the "granular" agglutination reaction. This then is a reversal of Tyler's (1941, 1942) view. He believed that the normal *Patiria* fertilizin was "univalent" and that the sperm was multivalent.

The various adjuvant solutions stimulate the sperm to intense motility and presumably expose more antifertilizin on the sperm surface. The latter effect is believed to be the essential one in rendering the sperm agglutinable. This action of the adjuvants bears a superficial resemblance to the "transformation" of human erythrocytes by an enzyme present in certain bacterial filtrates (Thomsen effect). Any human serum will agglutinate these transformed cells. There are several important differences between the process of erythrocyte transformation (Friedenrich, 1930) and the action on starfish sperm. The transformation requires a considerable period of time (15 minutes to several hours), is irreversible, and involves a fixation and subsequent release of the transforming principle. The action on *Patiria* sperm takes place very rapidly, the process reverses slowly when the adjuvant is removed, and it involves no fixation of the adjuvant. Repeated attempts failed to show any neutralization or absorption of egg white adjuvant by sperm or sperm-fertilizin mixtures. Friedenrich (1930) believes that a new agglutinogen is developed which is not present in latent or unreactive form on the normal erythrocyte. However, the case of *Patiria* sperm is more easily explained by assuming that a considerable amount of antifertilizin is in latent form on or near the cell surface.

Di Macco's (1923) "coagglutination" of sheep erythrocytes by mixtures of ricin and guinea pig serum also resembles the fertilizin-adjuvant agglutination of *Patiria* sperm. Neither ricin nor guinea pig serum alone agglutinated the sheep cells. Absorption of the separate solutions with cells failed to remove the active agents. Agglutination failed to occur if the ricin and guinea pig serum were mixed

first and the sheep cells added subsequently. Thus neither of the necessary agents reacted directly with the cells. Di Macco concluded that agglutination of sheep cells resulted from a reaction between the cells and an evanescent ricin-serum complex formed at a critical stage in the reaction between these substances. It is apparent, then, that the mechanism of the coagglutination is fundamentally different from the fertilizin-adjuvant agglutination of *Patiria* sperm.

The striking difference in fertilizing power of normal and adjuvant-treated sperm can be explained by the motility of the cells. Furthermore, this effect should be expected, regardless of motility, from the recent views of Tyler (1941). He has shown that fertilizin treatment lowers the fertilizing power of *Patiria* sperm and explained this by assuming that at fertilization a union occurs between antifertilizin on the sperm and fertilizin at the egg surface. If all of the sperm antifertilizin is bound by free fertilizin, then no reaction can occur between sperm and the surface of the egg. It follows from this that the normal univalent sperm would have much less chance of reaching the egg surface in an unsaturated condition than would the multivalent sperm. At present it is impossible to judge the relative importance of the intense motility and the multivalency of the adjuvant-treated sperm in this fertilization effect. If this increased fertilizing power should be found in species that regularly give low percentages of fertilized eggs, it might be useful for technical purposes.

Summary

I. Starfish sperm does not ordinarily agglutinate when treated with homologous fertilizin. However, when the sperm of some species (*Patiria miniata, Pisaster ochraceus, Astropecten* sp.) is treated with certain adjuvants the cells become intensely active and agglutinate when fertilizin is added. This reaction provides a means for studying the relationship between starfish sperm and fertilizin.

II. *Patiria* sperm will combine with homologous fertilizin and remove it from solution even in the absence of the adjuvant.

III. Cross tests between *Patiria, Pisaster* and *Astropecten* sperm and fertilizin solutions revealed no cross agglutination reactions.

IV. It is concluded that *Patiria* fertilizin is multivalent, since irradiated fertilizin will not agglutinate treated sperm but will inhibit the agglutination of such sperm by normal fertilizin; and since normal fertilizin will agglutinate sperm which has been freed of adjuvant.

V. It is suggested that normal *Patiria* sperm possesses but a single antifertilizin combining group and that more such groups are exposed on the sperm surface through the action of the adjuvant. Experiments which show that the fertilizin binding power of sperm is increased by the adjuvant support this view.

I am most grateful to Dr. Albert Tyler for the aid and encouragement he has given throughout the course of the work.

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COCHLIOPHILUS DEPRESSUS GEN. NOV., SP. NOV. AND COCHLIOPHILUS MINOR SP. NOV., HOLOTRICHOUS CILIATES FROM THE MANTLE CAVITY OF PHYTIA SETIFER (COOPER)

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Introduction

Examination of specimens of the pulmonate snail *Phytia setifer* (Cooper) [1] from salt marshes bordering San Francisco Bay disclosed the presence of two closely related species of flattened holotrichous ciliates within the mantle cavity. A new genus, *Cochliophilus,* is proposed to include these ciliates, which will be described herein as *Cochliophilus depressus* gen. nov., sp. nov. and *Cochliophilus minor* sp. nov.

I wish to express my appreciation to Professor S. F. Light and Professor Harold Kirby for their interest and helpful advice during the progress of this investigation.

Technique

Phytia setifer occurs under matted vegetation and debris in salt marshes and in the vicinity of brackish water ponds on the Pacific Coast of central and northern California. Material for this study was collected at several localities along the east shore of San Francisco Bay at Oakland and Berkeley.

For observation of the living ciliates the shell of the snail was carefully removed and the anterior part of the animal crushed in a drop of sea water on a slide. Fixation of the organisms for permanent preparations was accomplished by liberating them in this manner on a coverglass and then dropping the coverglass smear-side down onto the surface of the fixative in a Petri dish.

Staining with iron hematoxylin gave good results following the fixatives of Schaudinn, Champy, Bouin, and Heidenhain ("susa"). For a study of the ciliary system the method devised by Bodian (1936, 1937) for impregnation with activated silver albumose (protargol) was used after fixation in Hollande's cupric-picro-formol mixture. The Feulgen nuclear reaction was tried with success on material fixed in a saturated aqueous solution of mercuric chloride with 5 per cent of glacial acetic acid.

Description of Species

There is no agreement among protozoologists in regard to the orientation for descriptive purposes of compressed ciliates in which the cytostome is situated along the margin of the flattened body or displaced to the surface opposite that in

[1] Dall (1921) has implied that the species described by Cooper (1872) is distinct from *Phytia myosotis* (Drap.) of Europe and the Atlantic Coast of North America. No conclusive evidence has been presented to support or to refute this contention.

contact with the substrate. Hentschel (1924), writing of *Entodiscus* (*Cryptochilum*) *borealis*, stated that "since convention dictates that the side on which the mouth is situated shall be called ventral, we must say that the animal is flattened from side to side." Reichenow (1927–29) applied this scheme to *Conchophthirus*, as did also Kahl (1931, 1934) and Raabe (1932, 1934b).

De Morgan (1925), in his description of *Kidderia* (*Conchophthirus*) *mytili*, considered the concave under-surface to be ventral and the position of the cytostome to be lateral. Kidder (1933b) recognized the oral surface of *Kidderia mytili* as the "physiological ventral surface," but for purposes of clearness accepted De Morgan's plan of orientation. In the present paper I will follow De Morgan and Kidder in referring to that surface of the body most often found in contact with the substrate as ventral. The lateral margin on which the cytostome is situated will be referred to as the oral margin, and the opposite side as the aboral margin.

Cochliophilus depressus gen. nov., sp. nov. (*Figs. 1 and 2*)

The body outline as seen from the dorsal or ventral aspect is ovoid, often somewhat truncate at the posterior end. A view from the oral or aboral margin shows this ciliate to be much flattened, the ventral surface being slightly concave and the dorsal surface convex. In some individuals the curvature of the dorsal surface appears to be less regular than in others.

Twenty living individuals taken at random ranged from 70 μ to 107 μ in length and from 47 μ to 77 μ in width, averaging about 93 μ by 63 μ. The thickness varied from 11 μ to 16 μ. The relation of the length to the width is not the same in all specimens. Fixation of the organisms on coverglasses produced some shrinkage and frequently also distortion of shape due to compression.

The elongated peristomal area is situated in the posterior fourth of the body. Specialized ciliary elements which will be described presently extend from the anterior end of the peristomal indentation to the cytostome. That part of the peristomal area lying posterior to the cytostome is naked.

A well-defined pharynx is not present. I prefer to regard the irregular tubular structure which passes from the cytostome into the cytoplasm as the gullet. This gullet is difficult to see in living individuals, but in fixed material is demonstrable following staining in iron hematoxylin. As it approaches the macronucleus the gullet widens out and its boundaries become inexact.

A thin pellicle covers the body. Flexure of the pellicle in this ciliate is rarely noted, and then only when the animal comes in contact with solid obstructions in its path of movement. Trichocysts are absent.

The cilia of the body are disposed in 52 to 56 longitudinal rows and beat metachronously. The cilia on the dorsal and ventral surfaces are somewhat longer than those along the margin. The ventral cilia are thigmotactic, but not strongly so. On the ventral surface at the anterior end is a transverse suture (anterior field) from which the ventral rows of cilia extend backward, and from which the dorsal rows curve upward and continue posteriorly. Most of the dorsal rows converge in a characteristic pattern towards the posterior end. A definite unciliated area is evident between the longer dorsal rows and the ventral rows which curve upward a short distance over the posterior end.

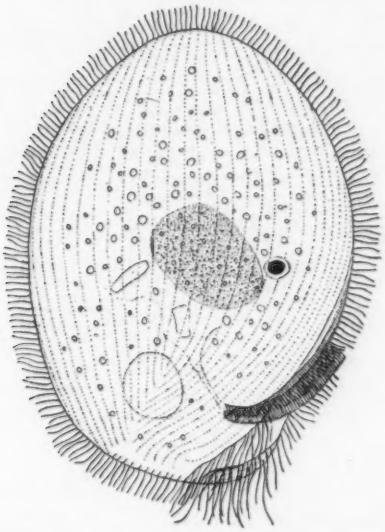

FIGURE 1. *Cochliophilus depressus* gen. nov., sp. nov. Dorsal aspect. Heidenhain's fixative ("susa")-iron hematoxylin. Drawn with aid of camera lucida. × 900.

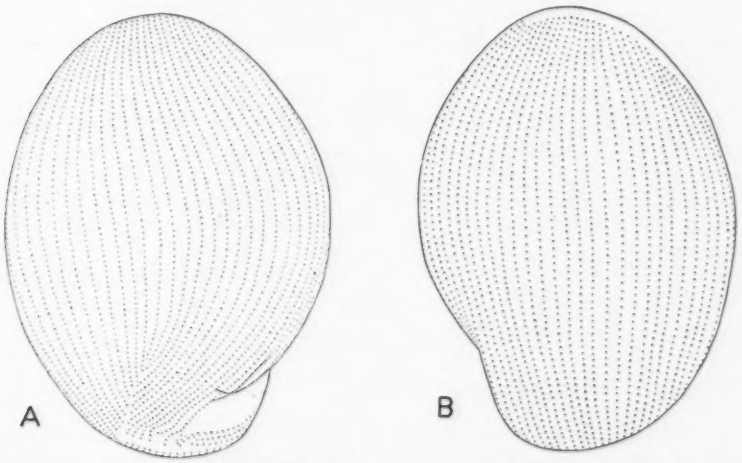

FIGURE 2. *Cochliophilus depressus* gen. nov., sp. nov. Distribution of ciliary rows. Hollande's fixative-protargol. Drawn with aid of camera lucida. *A*. Dorsal aspect. Though distorted somewhat due to compression, this individual shows well the arrangement of ciliary rows entering the peristomal indentation. × 670. *B*. Ventral aspect. × 950.

Three ventral rows of cilia close to the oral margin turn dorsally near the end of their course to delimit the naked part of the peristomal area posteriorly. The first of these rows is ordinarily seen to ramify into an incomplete double or triple series of cilia. The post-peristomal extensions of the ventral rows and the terminal part of the dorsal row which borders the peristome above bear cilia which are two to three times as long as the peripheral cilia elsewhere on the body.

The specialized peristomal cilia arise from two series of closely-set basal granules, each of which is seen to be a continuation of two rows of peripheral cilia essentially lateral in position, lying between the three ventral rows of cilia and one dorsal row marking off the peristomal area. The cilia of the upper peristomal row are appreciably longer than those of the lower row and appear in living individuals to form a membrane-like structure which beats up and down as a unit. The cilia of the lower row are much thicker and do not beat synchronously. The activity of the peristomal cilia ceases soon after the organism is dissociated from the host.

The cytoplasm is colorless. Greenish granules appearing as highly refractile bodies are distributed through the cytoplasm. These are most numerous around the macronucleus and following fixation stain intensely with iron hematoxylin.

The macronucleus is centrally located. In outline it varies from oblong to round, and in life is conspicuous as a clear granular body surrounded by food inclusions and cytoplasmic granules. The micronucleus is greenish in color and difficult to detect in living individuals. It is easily demonstrated by iron hematoxylin or the Feulgen nuclear reaction. The micronucleus is commonly situated close to the macronucleus, between the latter and the oral margin. Upon fixation it shrinks considerably and draws away from the membrane by which it is invested.

The contractile vacuole lies in the posterior fourth of the body behind the gullet, and apparently opens to the exterior at a point between the convergence of the shorter dorsal ciliary rows. I have been unable to distinguish a permanent opening in the pellicle.

When free in water, *Cochliophilus depressus* swims actively, generally in circles and with its concave ventral surface in contact with the substrate. Occasionally, however, it follows an erratic course, rotating on its longitudinal axis. The transverse anterior field is always at right angles to the direction of movement. In the presence of pieces of tissue from the host *Cochliophilus depressus* will sometimes seek refuge among them or cling to epithelial surfaces by means of its ventral thigmotactic cilia.

I have found *Cochliophilus depressus* to be present in the mantle cavity of nearly all specimens of *Phytia setifer* which I have examined. It occurs in small numbers and is usually less common than the following species.

Cochliophilus minor sp. nov. (*Figs. 3 and 4*)

The shape of this species resembles in general that of *Cochliophilus depressus*, except that the posterior end is rather pointed, never truncate, and the dorso-ventral dimension in relation to the length and breadth is comparatively greater. In addition, the curvatures of the ventral and dorsal surfaces are more pronounced in *Cochliophilus minor*.

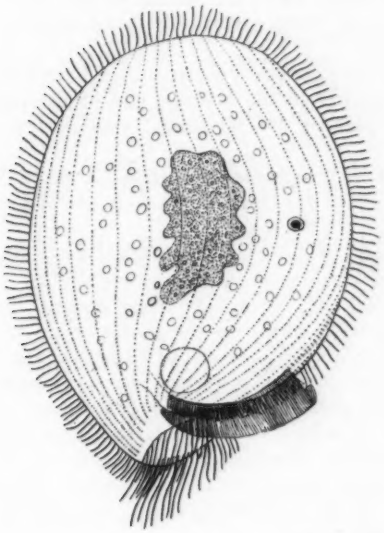

FIGURE 3. *Cochliophilus minor* sp. nov. Dorsal aspect. Heidenhain's fixative ("susa")-iron hematoxylin. Drawn with aid of camera lucida. × 1250.

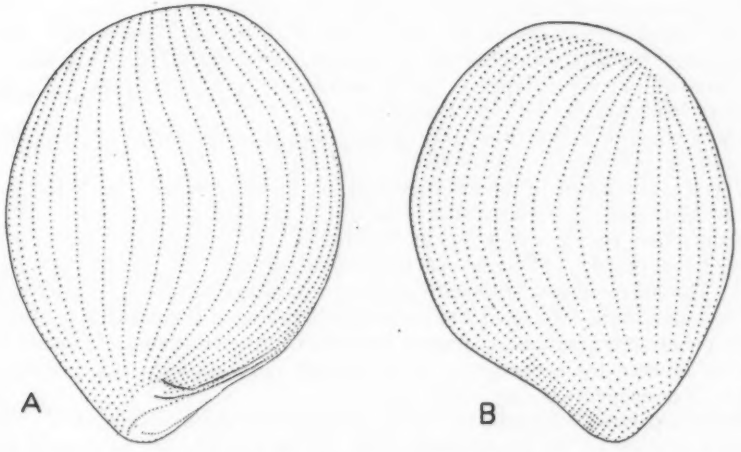

FIGURE 4. *Cochliophilus minor* sp. nov. Distribution of ciliary rows. Hollande's fixative-protargol. Drawn with aid of camera lucida. *A*. Dorsal aspect. × 1250. *B*. Ventral aspect. × 1250.

Twenty living individuals taken at random ranged from 51 μ to 80 μ in length and from 33 μ to 56 μ in width, averaging about 63 μ by 45 μ. The thickness varied from 11 μ to 18 μ.

The peristomal area is situated in the posterior fourth of the body. Two rows of specialized cilia extend from the anterior end of the peristomal indentation to the cytostome. That part of the peristomal area posterior to the cytostome is naked.

An irregular gullet may sometimes be traced a short distance from the cytostome, but it is not as easily discerned as the comparable structure in *Cochliophilus depressus*.

The cilia are disposed in 36 to 38 longitudinal rows and beat metachronously. The cilia on the ventral and dorsal curvatures are slightly longer than those along the margin. The ventral cilia are weakly thigmotactic. The ventral rows extend from an anterior transverse suture to the posterior tip of the body. The basal granules of most of the ventral rows come to lie farther apart towards the posterior end, while those of three or four rows near the oral margin lie closer together. The dorsal rows of cilia pass from the transverse suture over the anterior end of the body and continue backward to terminate in a conformation homologous with that found in *Cochliophilus depressus*. The posterior dorsal unciliated area of *C. depressus* has no exact homologue in this species. There exists, nevertheless, an unciliated area between the converging dorsal rows and the dorsal row bordering the peristomal area above.

One or two rows of cilia following the oral margin curve dorsally near the end of their course to delimit the naked part of the peristomal area posteriorly. These extensions and the terminal part of the most nearly lateral dorsal row on the oral side bear exceptionally long cilia.

The peristomal ciliary apparatus consists of a membrane-like structure of long, fine cilia which curves downward over a row of closely-set, rather thick cilia extending from the anterior end of the peristomal indentation to the cytostome. The membrane-like structure appears to be non-motile and to function as a funnel directing food particles into the cytostome.

The cytoplasm is colorless. Refringent cytoplasmic granules are present, but to a lesser extent than in *Cochliophilus depressus*.

The size and shape of the macronucleus are highly variable. In living as well as fixed individuals it is nearly always seen to be ramified, although ovoid or round macronuclei are occasionally noted in this species. Reorganization stages in which two or more smaller and round macronuclei are present are not infrequently met with. The micronucleus ordinarily occupies a position between the macronucleus and the oral margin. In fixed and stained preparations it is considerably shrunken.

The contractile vacuole is situated anterior to the cytostome. It opens to the exterior between the convergence of the shorter dorsal ciliary rows. I have not detected a permanent opening in the pellicle.

When separated from its host *Cochliophilus minor* swims in circles or proceeds forward rotating on its longitudinal axis. Its movements are in general slower than those of *Cochliophilus depressus*.

Cochliophilus minor is found in association with *Cochliophilus depressus* in the mantle cavity of *Phytia setifer*. It is usually more numerous than *C. depressus*.

Systematic Position

On the basis of certain features of the morphology of the two species of *Cochliophilus* which I have described it may be justifiable to allocate this genus to the sub-order *Thigmotricha* Chatton and Lwoff, although in view of the deficiencies of the systems of classification of holotrichous ciliates currently recognized I must defer a conclusive statement with regard to its position. The organization of the peristome of *Cochliophilus* hints its affinity with *Kidderia* Raabe, represented by *K. mytili* (De Morgan) from *Mytilus edulis*. Raabe (1936) retained *Kidderia* in the family Conchophthiridae Reichenow,[2] but removed to the family Thigmophryidae Chatton and Lwoff *Myxophyllum* and *Conchophyllum*, genera created by him to accommodate, respectively, Stein's species *Conchophthirus steenstrupi*, commensal on various terrestrial pulmonate molluscs, and *Conchophthirus caryoclada* Kidder, from the bivalve *Siliqua patula*. It is interesting to note, in passing, that a specific character of *Conchophyllum caryoclada* is its branched macronucleus, of which the macronucleus of *Cochliophilus minor* is reminiscent.

The presence of a membrane-like structure in the peristome of *Cochliophilus* could be the basis for objections to the inclusion of this genus in the Thigmotricha. Very similar ciliary elements have been observed, however, in certain species of the family Ancistrumidae Issel. Raabe (1932, 1934b) has stressed the presence of an undulating membrane in *Conchophthirus*, although Kidder (1934), after studying species of *Conchophthirus* from fresh water mussels in this country, was unable to corroborate Raabe's findings, and suggested that Raabe may have mistaken the fibers of the peristomal basket for an undulating membrane.

Genus Cochliophilus gen. nov.

Diagnosis: Flattened holotrichous ciliates, ovoid in outline as seen in dorsal or ventral view. The peristomal area is elongated and is situated on the right lateral margin in the posterior fourth of the body. A membrane-like structure of fine cilia overlies a series of thick cilia extending from the anterior end of the peristomal indentation to the cytostome; that part of the peristomal area posterior to the cytostome is naked. The peripheral cilia are disposed in longitudinal rows extending from a ventral transverse suture at the anterior end of the body. The dorsal rows converge in a characteristic pattern posteriorly. Thichocysts are absent. The macronucleus is centrally located; the micronucleus is usually situated near the macronucleus, between the latter and the oral margin. The contractile vacuole opens to the exterior between the convergence of the shorter dorsal ciliary rows; no permanent opening in the pellicle is discernible. Genotype: *Cochliophilus depressus* gen. nov., sp. nov. Two species, commensal in the mantle cavity of *Phytia setifer* (Cooper).

Cochliophilus depressus gen. nov., sp. nov.

Diagnosis: Average size about 93 μ by 63 μ, the thickness being about one-sixth the length. The ciliary rows are 52 to 56 in number. The peristomal membrane-like structure is motile. The macronucleus is round or oblong. Syntypes are in the collection of the author.

[2] Reichenow (1927–29) was apparently the first to use the name Conchophthiridae, although Raabe credits Kahl (1931) with establishing this family.

Cochliophilus minor sp. nov.

Diagnosis: Average size about 63 μ by 45 μ, the thickness being about one-fourth the length. The ciliary rows are 36 to 38 in number. The peristomal membrane-like structure is apparently immobile, serving as a funnel directing food particles into the cytostome. The macronucleus is characteristically ramified. Syntypes are in the collection of the author.

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THE DEVELOPMENT OF MARINE FOULING COMMUNITIES

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This paper constitutes an examination of the sedentary communities found on float bottoms and other submerged objects in Newport Harbor, California. Particular attention has been paid to the changes in composition of such communities with time.

The basic problem in the development of a sequence of communities in a limited environment is that of distinguishing between seasonal progression and true succession. Seasonal progression results fundamentally from differences in breeding seasons of various organisms. This type of development was noted at Beaufort, N. C. by McDougall (1943). Most of the organisms observed by McDougall had short life cycles and short breeding seasons. As a result, most of the organisms which settled in the winter months were dead or moribund by spring, and were replaced by organisms breeding in the latter season.

Succession, in contrast to seasonal progression, involves definite relations between organisms, Shelford (1930) has suggested the following criteria for the occurrence of succession: (1) Early forms must drop out, and be replaced by later forms, and (2) Some of the earlier forms must be essential for the establishment of the later forms. The use of the word "essential" in this connection is perhaps unfortunate. It would be nearly impossible, in most cases, to prove that one organism is essential for the establishment of another. On the other hand, the presence of one organism might well provide conditions favoring the establishment of another, and certainly such favorable conditions would suffice to insure the displacement of early settlers by later arrivals.

The phenomena of ecological succession are well known in terrestrial communities. In littoral marine communities, it has sometimes been stated that true succession does not occur, or is of little importance (Shelford, 1930; McDougall, 1943). The clearest case of succession in intertidal communities is that reported by Hewatt (1935). In the *Mytilus californianus* community characteristic of exposed rocky coasts along the entire Pacific coast of the United States, the establishment of a climactic condition requires more than two and one-half years, and involves a definite sequence of organisms. The reports of Kitching (1937), Moore (1939) and Moore and Sproston (1940) also give some indication that recolonization of intertidal rock surfaces is a slow process. It appears that the first event is ordinarily a heavy settlement of algae, and that many animal forms appear only after the plants have become established. Kitching (1937) provides evidence of a succession of algal forms on rocky intertidal ledges.

The sedentary organisms inhabiting floats, pilings, boat bottoms and similar structures have been the subject of many investigations. The literature in this field has been reviewed recently by McDougall (1943) and need not be cited ex-

tensively here. The most thorough investigations dealing with the Pacific forms are those of Coe (1932) and Coe and Allen (1937). These studies, covering a period of nine years, have provided invaluable information regarding the biology of the organisms concerned. The data reported in the current study have been accumulated between February 1943 and March 1945.

The Float-Bottom Communities of Newport Harbor

Field observations on float bottoms and similar structures in Newport Harbor disclosed the existence of five or six rather definite communities. For convenience, throughout this paper, these communities will be referred to by designations indicating the most abundant organisms in the community. In this way, we may designate (a) algal, (b) bryozoan, (c) *Ciona,* (d) *Styela,* (e) *Mytilus,* and (f) *Balanus* communities. These communities were not all sharply marked off, one from another, and communities intermediate in composition between algal and bryozoan, bryozoan and *Styela, Styela* and *Mytilus,* bryozoan and *Mytilus,* and *Ciona* and *Mytilus* have been observed. The various communities showed no relation to the position of the floats in the harbor, and indeed several different communities were found within a distance of a hundred feet on different floats. Evidence will be presented that this results from a definite succession, and that the composition of the community on any particular float bottom depends on (a) the length of time during which the float has been in the water, and in part on (b) the season during which the float was first immersed. We shall first consider the composition of the various communities.

The bottoms of floats were examined with the aid of a periscopic device involving an ordinary underwater viewing glass with a mirror attached (Fig. 1). Organisms were also removed from floats with a long-handled scraper.

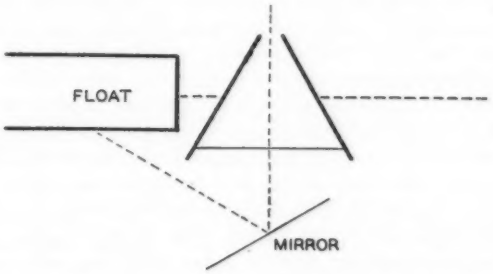

Figure 1. Apparatus for the examination of float bottoms.

The algal community. When a clean surface was placed in the bay, the first settlers were bacteria, algae, protozoans, and, during the cooler months of the year, hydroids. The algae included small sedentary diatoms which have not been identified in the present study (see Coe, 1932; and Coe and Allen, 1937), colonial diatoms of the genus *Licmophora,* and one or more species of *Ectocarpus,* notably *E. granulosoides.* In addition, *Enteromorpha* sp., *Lophosiphonia villum,* and *Pterosiphonia bipinnata* were frequently noted. The sedentary protozoans in-

cluded a form similar to *Zoothamnium,* and the suctorian *Ephelota.* There were seven or eight species of hydroids; these were not identified, but *Obelia dichotoma* was usually conspicuous. Bryozoans were found in this community, sometimes in abundance. On float bottoms, *Bugula neritina* may be an important member of the community, and *Membranipora tuberculata* was observed in one instance on glass plates. *Eucratea clavata,* a small semi-erect bryozoan, occasionally occurred in considerable numbers on glass plates. Finally, young colonies of a number of other species of bryozoans appeared after a time. These will be discussed in more detail later.

The bryozoan community. A good many floats supported a very heavy growth of bryozoans. The principal organisms involved were the encrusting bryozoans *Schizoporella unicornis, Cryptosula pallasiana, Rhynchozoon tumulosum* and *Holoporella aperta.* The erect bryozoans were less constant in occurrence, but were quite abundant in some cases. *Bugula neritina* was less frequent in this community than among the algae, while *Eucratea clavata* was more frequently found among the encrusting bryozoans than among the algae. *Crisulipora occidentalis* and *Scrupocellaria diegensis* were usually present and often very abundant among the bryozoans. Four or five other species of erect bryozoans occurred less frequently.

Although the bryozoans by far outnumbered the other members of this community in most cases (Table VII), other organisms were often quite abundant. Notable are the serpulid worm *Eupomatus gracilis,* and the colonial amphipod *Erichthonius braziliensis. Eupomatus* was almost always found, with its winding calcareuos tubes, between the colonies of encrusting bryozoans. Occasionally, it was very abundant, the tubes making a more or less solid mass. *Erichthonius* was irregular in occurrence. During 1943, it did not appear in quantity, but in 1944 it was extremely abundant during July and August, the mud tubes often covering as much as half of the area of a glass plate. Coe and Allen (1937) noted a similar variation at La Jolla. The ascidians *Styela barnharti, Halocynthia johnsoni,* and *Ciona intestinalis,* and the mussel *Mytilus* sp. were found among the bryozoans in many cases, but since they were more characteristic of other communities, they will be dealt with later. Many crustaceans, annelids and other motile forms used the bryozoan clumps for shelter.

The Ciona community. The previous paragraphs have dealt with communities in which several species were abundant and the proportions of each species showed considerable variation in different communities of the same type. Most of the *Ciona* communities, in contrast, were composed almost wholly of specimens of *Ciona intestinalis.* This was particularly true during the summer and fall, when these communities were at their peak of development. Many float bottoms presented a solid mass of *Ciona,* with only a few other organisms present. These latter were usually colonial ascidians, growing on the tests of the *Ciona,* and such crustaceans and annelids as might have taken refuge among the stalks.

The Styela community. This was a poorly defined community, intermediate in composition between the bryozoan and *Mytilus* communities. The encrusting bryozoans noted earlier were usually present, forming a substratum for the stalks of *Styela,* while the erect bryozoans were often found among these stalks. Small specimens of *Mytilus* were often attached to the stalks in large numbers. Large

sponges, which have not been identified, were also frequently present, sometimes in such quantity as to dominate the community. It might indeed be preferable to refer to a *Styela*-Sponge community.

The Mytilus community. *Mytilus* was without question the most abundant dominant on the float bottoms in Newport Bay during the period of this study. This has not always been the case, according to reliable observers (G. E. MacGinitie, A. M. Strong, personal communications); during several previous years, *Mytilus* has not been abundant in the bay. The exact identity of this mussel remains in doubt. It is probably the same form which has been recorded infrequently from this area as *M. edulis*. However, conchologists are not entirely agreed that this is the proper designation. It is certainly not *M. californianus*. The *Mytilus* communities sometimes were observed on a substratum of old and badly decayed bryozoans; at other times they were attached directly to the float bottom. Old specimens of *Styela* or *Ciona* were often present among the mussel clumps, and various types of sponge were often quite abundant.

The Balanus community. Communities in which *Balanus* is the dominant organism were not observed on float bottoms in Newport Harbor, although they are frequently observed on experimental surfaces exposed in the open sea at La Jolla. Indeed, *Balanus tintinnabulum* is probably the principal dominant at La Jolla (Coe, 1932). One experimental panel exposed at this laboratory developed a *Balanus* community comparable to those observed at La Jolla, however.

Changes in Float-Bottom Communities

Eight floats, all located along the mainland side of the channel between Balboa Island and Corona del Mar, and within a distance of 100 yards of one another, were selected in September of 1944, and kept under observation for a period of six months. The results of this study are presented in Table I. At intervals of about one month, the bottom of each float was examined with the viewing glass, and samples of the population removed by hand and with the scraper for later examination in the laboratory.

Float number one had been immersed in the bay for only about one week previous to the first examination. It had at that time (Sept. 21) a typical algal community, with a few specimens of *Bugula*. In October, examination showed increased numbers of *Bugula*, and a few small colonies of other erect bryozoans. In November, *Bugula* and the encrusting bryozoan *Holoporella* had displaced the algae, and a number of small specimens of *Ciona* were present. The float was then covered with a typical bryozoan community. During the remainder of the period, until March, the encrusting bryozoans continued to increase in numbers and size.

Floats 2 and 3 supported typical bryozoan communities in September. In addition to the bryozoans, a number of specimens of *Ciona* were observed, and several small *Mytilus*. During the period of observation, *Mytilus* grew at the expense of the bryozoans and ascidians, becoming very abundant in December, and largely dominating the community by February. The two float populations were very similar in composition in September, but the presence of *Styela* on float 3 in October appears to have favored the earlier establishment of *Mytilus* on this float. The presence of sponges on this float may also be related to *Styela*. Float 4, in Sep-

TABLE I

Changes in composition of float-bottom populations, September 1944 to March 1945

Float number		September 21, 1944	October 23, 1944	November 22, 1944	December 21, 1944	February 13, 1945	March 26, 1945
1	Dominants	Algae	Algae	Bugula Holoporella	Bugula Holoporella	Encrusting Bryozoans	Encrusting Bryozoans
	Sub-dominants		Bugula	Ciona	Ciona		
	Influents	Bugula	Erect Bryozoans Ciona	Encrusting Bryozoans Eupomatus	Encrusting Bryozoans Eupomatus	Scrupocellaria Hydroids	Scrupocellaria Hydroids
2	Dominants	Encrusting Bryozoans Eupomatus	Encrusting Bryozoans Eupomatus	Encrusting Bryozoans Eupomatus	Mytilus Encrusting Bryozoans	Mytilus Encrusting Bryozoans	Mytilus Encrusting Bryozoans
	Sub-dominants	Ciona Bugula Scrupocellaria	Ciona Bugula Scrupocellaria	Ciona Mytilus	Ciona Erect Bryozoans	Erect Bryozoans	Erect Bryozoans
	Influents	Colonial Ascidians Mytilus Sponges	Colonial Ascidians Mytilus Sponges	Colonial Ascidians Sponges	Colonial Ascidians		
3	Dominants	Encrusting Bryozoans Eupomatus	Encrusting Bryozoans Eupomatus	Encrusting Bryozoans Eupomatus	Encrusting Bryozoans Mytilus	Mytilus	Mytilus
	Sub-dominants	Ciona Bugula Scrupocellaria	Ciona Styela Scrupocellaria	Styela Mytilus	Sponges	Sponges	Sponges
	Influents	Colonial Ascidians Mytilus Sponges	Colonial Ascidians Mytilus Sponges	Colonial Ascidians Sponges	Colonial Ascidians		
4	Dominants	Encrusting Bryozoans	Mytilus Ciona	Mytilus	Mytilus	Mytilus	Mytilus
	Sub-dominants	Ciona Mytilus		Ciona	Encrusting Bryozoans		
	Influents	Colonial Ascidians Erect Bryozoans	Colonial Ascidians Erect Bryozoans	Colonial Ascidians Erect Bryozoans			

TABLE I—*Continued*

Float number		September 21, 1944	October 23, 1944	November 22, 1944	December 21, 1944	February 13, 1945	March 26, 1945
5	Dominants	Ciona	Ciona	Ciona	Ciona	Ciona	Mytilus Encrusting Bryozoans
	Sub-dominants					Algae Hydroids	Ciona
	Influents					Mytilus Encrusting Bryozoans	Hydroids Colonial Ascidians
6	Dominants	Sponges	Sponges	Sponges Mytilus	Mytilus Sponges	Mytilus	Mytilus
	Sub-dominants	Styela Mytilus	Styela Mytilus	Styela		Sponges	Sponges
7	Dominants	Styela Mytilus	Mytilus	Mytilus	Mytilus Sponges	Mytilus	Mytilus
	Sub-dominants		Styela	Sponges			
	Influents	Bryozoans Sponges Pecten	Bryozoans Sponges Pecten	Bryozoans Pecten			
8	Dominants	Mytilus	Mytilus	Mytilus	Mytilus	Mytilus	Mytilus
	Influents	Styela Sponges	Styela Sponges	Styela Sponges	Styela Sponges	Styela Sponges	Styela Sponges

tember, had a population similar to that observed on float 2 in November, with relatively large numbers of *Mytilus* and *Ciona* on a bryozoan substratum. Within a month, *Mytilus* had largely displaced the bryozoans, and within three months, *Ciona* had also disappeared.

The *Ciona* community of float 5 remained virtually unchanged from September to February. By this time, however, the *Ciona* began to show signs of deterioration. They were heavily covered with algae and hydroids, and had many small *Mytilus* about their bases. In a few places, the ascidians had fallen from the float, to be replaced by encrusting bryozoans. In March, this change had progressed so far that *Mytilus* and the bryozoans could be regarded as the dominant organisms.

Floats 6 and 7 supported two types of sponge-*Styela* communities. These were rather rapidly displaced by *Mytilus*, however. Float 8 represented a well-developed *Mytilus* community and showed no change in composition during the six months of regular observation.

These observations suggest strongly that succession is operating here. The algal community is replaced by the bryozoans, and these in turn by *Mytilus*. *Ciona* and *Styela* communities are likewise replaced by *Mytilus*, but the *Mytilus* community is relatively stable. Further information bearing on this conclusion is available from the experimental studies to be reported in the next section.

Experimental Observations With Glass and Metal Surfaces

Experimental observations were made using glass plates, and supplementary information was available from a series of aluminum panels immersed for another purpose. The fact that the changes observed on the glass plates were entirely similar to those observed on wooden floats and metal plates suggests that the changes reported here are not dependent on the nature of the submerged surface. Coe (1932) and Coe and Allen (1937) concluded that the seasonal variations in abundance of populations or of different groups of organisms were the same on glass, concrete, and wood surfaces. They did find significant differences in the numbers and types of organisms on the different surfaces, however.

The glass panels used were four by nine inch rectangles of ordinary window glass in most cases; in a few experiments three by five inch panels were used. The metal plates were five by eight inch rectangles of aircraft aluminum (Alclad ST-37). The glass panels were at first exposed in a horizontal frame (Fig. 2) of redwood weighted with concrete. The frame was suspended from the laboratory pier, situated in the entrance channel to Newport Harbor about one-half mile from the outer end of the jetties protecting the harbor entrance. A rapid tidal flow passes this point twice daily, carrying with it abundant larvae from both the quiet-water fauna of the harbor and the open shore fauna of the jetties and adjacent rocks. In the second year of this study, with the glass plates, and throughout the work with the metal plates, a vertical suspension was used to facilitate handling of larger numbers of plates. The plates were suspended in slotted redwood crates, with a distance of one inch between plates. As the growth on the plates became heavier, this distance was increased to two inches. The plates were always suspended one or two feet below the level of the lowest tides.

All of the plates were examined regularly at intervals of two weeks, and then returned to the bay. A count was made, in most instances, of the numbers of each of the larger species on one surface (always the same for any plate). An estimate was also made of the area covered by each of the more abundant types of organism. Usually, this was done by a direct count of ten or more low-power microscopic fields distributed over the surface. When a plate was finally removed from the water, the organisms were carefully removed, sorted and weighed.

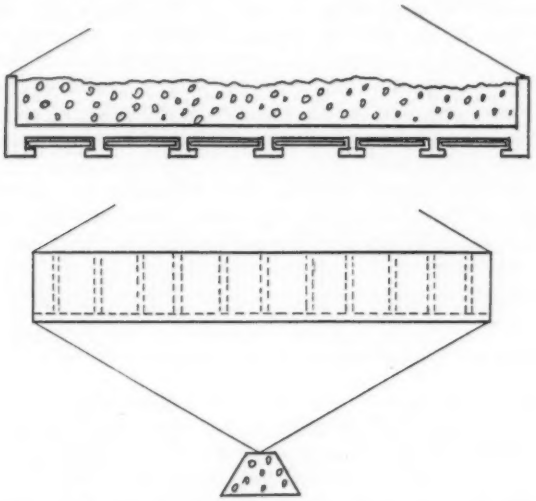

FIGURE 2. Horizontal and vertical suspension of panels.

The development of the algal community, and its transition to the bryozoan community could be followed very well on these plates. The first settlers were bacteria, diatoms, protozoans and, in the cooler months, hydroids. These were followed by the multicellular algae, especially *Ectocarpus*.

In the first months of this study it was observed that the larvae of bryozoans usually settled on the plates in quantity only after the second week of exposure, and sometimes did not settle until the fourth to sixth week. In order to verify this observation, careful counts were made during 1944 of the number of bryozoan colonies on each plate at two-week intervals. In this way, the minimum number of new settlers during any two-week period could be determined. Data obtained in this way are tabulated in Tables II, III, and IV for encrusting bryozoans, erect bryozoans and *Eupomatus*. The tabulation for the erect bryozoans omits the figures for the small semi-erect *Eucratea clavata;* representatives of this species settled in great numbers at irregular intervals, showing a behavior in this respect which was not at all comparable to the settlement of the other forms. The colonies were, moreover, rather short lived, dying often within a month of the original settlement.

During the first two weeks of exposure of any plate, the number of bryozoan and tubeworm settlements was usually less than during subsequent two-week periods. The preliminary period of light settlement was followed by a very heavy settlement in most cases. The growth of the early settlers, and in many cases, the large number of organisms settling during the maximal period, combined to reduce the available surface, and there was in consequence a very definite decrease in the number of organisms on the plate. By the time this decrease became evident, the plate was completely covered with bryozoans and tubeworms.

TABLE II

Number of new settlements of encrusting bryozoans on glass plates during successive two-week periods, 1944

Date examined	Date of original exposure														
	Dec. 20	Jan. 5	Jan. 17	Jan. 31	Feb. 14	Feb. 28	Mar. 12	Mar. 27	Apr. 27	May 9	June 8	July 6	Aug. 1	Sept. 11	Oct. 10
Jan. 5	0														
Jan. 17	0	0													
Jan. 31	0	0	0												
Feb. 14	3	0	0	0											
Feb. 28	10	1	0	0	2										
Mar. 12	28	5	2	3	—	0									
Mar. 27	30	26	30	14	5	0	0								
Apr. 8	0	7	—	18	8	10	2	2							
Apr. 26	24	62	60	120	64	60	57	9							
May 8					47	46	60	123	61						
May 24							12	13	17	14					
June 7									35	94					
June 21									11	53	30				
July 6											23				
July 17											48	25			
July 31											80	197			
Aug. 14												47	7		
Aug. 28												50	105		
Sept. 11												15	54		
Sept. 25													22	24	
Oct. 10														34	
Oct. 23														10	11

Figure 3 represents data derived from a metal plate first exposed March 28, 1944, and shows the changes in area covered by the algae, bacteria and hydroids on the one hand, and bryozoans on the other. The major increase in area occupied by the bryozoans occurred after the period of maximum settlement; the heaviest settlement occurred between the sixth and eighth weeks, while the rapid increase in area began between the tenth and twelfth weeks. This was in part the result of the manner of growth of bryozoan colonies. The number of new zooids formed increases directly with the number of zooids composing the colony, so that the rate of growth increases exponentially until crowding prevents further increase in the size of the colony.

TABLE III

Number of new settlements of erect bryozoans (exclusive of *Eucratea clavata*) on glass plates during successive two-week periods, 1944

Date examined	Date of original exposure												
	Jan. 17	Jan. 31	Feb. 14	Feb. 28	Mar. 12	Mar. 27	Apr. 27	May 9	June 8	July 6	Aug. 1	Sept. 11	Oct. 10
Mar. 27	0	0	0	0	0								
Apr. 8	4	0	0	0	0	0							
Apr. 26	11	10	10	0	5	0							
May 9	20	29	28	35	48	3	0						
May 24	5	4	6	—	17	18	0	0					
June 7	11	18	7	22	14	17	5	10					
June 21	21	27	36	24	—	27	72	44	2				
July 6								40	5				
July 17									28	12			
July 31									33	3			
Aug. 14									28	11	1		
Aug. 28										13	4		
Sept. 11											9		
Sept. 25											19	1	
Oct. 10											18	0	
Oct. 23													1

The length of time required for this sequence of events varied with the season of the year, but the character of the sequence did not vary. Thus, the plate exposed December 20 did not reach "saturation" with encrusting bryozoans until April, while the plate exposed May 9 had become "saturated" before the end of June (Table II). If we consider any particular two-week period, however, it is

TABLE IV

Number of new settlements of *Eupomatus* on glass plates during successive two-week periods, 1944

Date examined	Date of original exposure														
	Dec. 20	Jan. 5	Jan. 17	Jan. 31	Feb. 14	Feb. 28	Mar. 12	Mar. 27	Apr. 27	May 9	June 8	July 6	Aug. 1	Sept. 11	Oct. 10
Jan. 5	0														
Jan. 17	0	0													
Jan. 31	0	0	0												
Feb. 14	2	0	0	0											
Feb. 28	15	4	0	0	0										
Mar. 12	17	10	1	0	0	0									
Mar. 27	7	1	6	2	0	0	0								
Apr. 8	8	14	13	9	1	2	0	0							
Apr. 26	1	1	10	2	4	2	0	0							
May 8	1	3	2	—	0	1	1	0	0						
May 24					2	—	—	0	0	0					
June 7							3	1	2	0					
June 21							0	3	0	7	8				
July 6							7	4	12	20	11				
July 17											4	0			
July 31											2	5			
Aug. 14											—	5	0		
Aug. 28											14	39	15		
Sept. 11													11		
Sept. 25													—	11	
Oct. 10													4	3	
Oct. 23														3	0

evident from Tables II to IV, that in general, the most recently exposed plates received lighter settlements of the three types of organisms concerned than did those which had been in the water somewhat longer. Evidently changes occurred following immersion which rendered the plate more suitable for settlement of bryozoans and tubeworms than was the clean surface. These changes occurred more rapidly in the warmer months.

Two experiments were performed to test this hypothesis, and to throw more light on the nature of the changes involved. ZoBell and Allen (1935) and Coe and

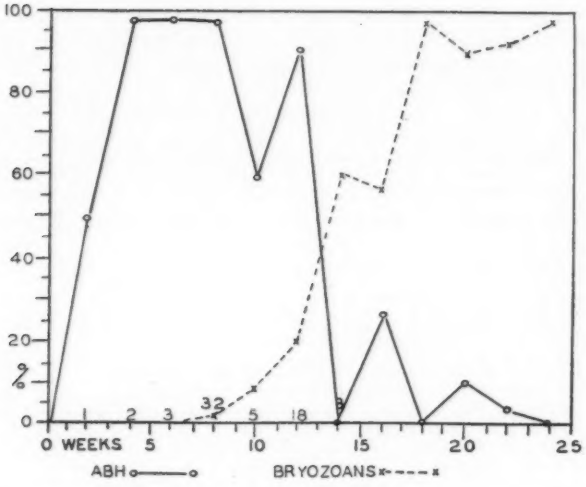

FIGURE 3. Relative areas, in per cent, covered by algae, bacteria, and hydroids (A B H), and bryozoans on an aluminum panel exposed March 28, 1944. The figures along the abscissa represent number of new settlements of bryozoans in each two-week period.

Allen (1937) have suggested that bacterial film is an important feature in the establishment of sedentary forms on a submerged surface. In the first experiment (Table V), ten three by five inch glass plates were sterilized. Two were then exposed in the bay, two were left in sterile sea water, two in a sterile solution of 0.1 per cent peptone in sea water, and two were placed in a solution of 0.1 per cent peptone in water freshly drawn from the bay. After four days, by which time a vigorous bacterial population had developed in the bay water solution, all ten

TABLE V

Settlement of organisms on pretreated glass plates, June 6–10, 1944. Duration of treatment, 4 days. Figures represent number of organisms or colonies

Treatment:	First series (3 days immersion)			Second series (4 days immersion)		
	Hydroids	Bryozoans	Ascidians	Hydroids	Bryozoans	Ascidians
Sterile sea water	43	0	0	68	4	0
Sterile sea water + 0.1% peptone	53	2	2	44	0	0
Bay water + peptone (bacterial)	150	2	2	174	4	0
Immersal in bay 4 days	53	11	11	83	14	0
Sterile plate	47	2	2	52	3	0

plates were placed in the bay. The results are presented in Table V. The hydroids evidently settled more abundantly on the bacteria-coated plates then on the others, but the bryozoans and ascidians were not influenced by the bacterial coating. Rather, they settled more abundantly on the plates which had been in the bay longest; these plates had a more abundant diatom population than did the others.

A second similar experiment was carried out in the fall, with daily observations during several weeks of exposure, and careful determinations of the bacterial and algal populations. Diatoms appeared on the plates in small numbers within the first two to four days in the bay (Table VI). For a period of two to three weeks, however, the diatoms covered less than 5 per cent of the surface. This period was

TABLE VI

Settlement of organisms on treated panels, October 21 to November 24, 1944. The figures represent the per cent of the area of one side of the panel covered by bacteria, diatoms, and protozoans respectively, and total number of organisms or colonies on one side of the panel for the larger organisms (bryozoans, annelids, ascidians).

Duration of treatment:		5 days				18 days			
Treatment:		Bay	Sterile	Sterile sea water + peptone	Fresh bay water + peptone	Bay	Sterile	Sterile sea water + peptone	Fresh bay water + peptone
Days after treatment	Organism								
7	Bacteria	0.3%	0.2%	0.3%	1%		1%	2%	11%
	Diatoms	4%	1%	1%	1%	80%	0.3%	1%	1%
	Protozoans	+	+	+	+		0.3%	1%	+
	Bryozoans:								
	Membranipora	6	3	1	9	0	1	0	0
	Other encrusting forms	0	0	2	0	8	1	2	3
	Annelids	0	1	0	3	3	0	0	8
	Ascidians	1	0	5	3	0	0	3	7
16	Bacteria	1%	1%	2%	1%		5%	7%	8%
	Diatoms	35%	11%	8%	16%	51%	5%	4%	18%
	Protozoans	6%	1%	0.1%	6%		3%	5%	6%
	Encrusting bryozoans exc. Membranipora	13	6	11	14	14	8	9	17
	Annelids	1	2	2	3	8	0	1	9
	Ascidians	2	2	9	5	0	2	1	0
Time of maximum increase, days after immersion in bay	Diatoms	19	14	20	14	19	16	14	16
	Encrusting bryozoans exc. Membranipora	19	14	22	16	21	16	16	16

followed by a relatively sudden increase in the number of diatoms, until 25 per cent to 80 per cent of the plate was covered. The reason for this delay is not clear. During the first two to three weeks of exposure, bacteria and protozoans as well as diatoms settled on the plates. That bacteria were not concerned in the eventual diatom outburst is indicated by the fact that the bacteria-coated plates (bay water and peptone) showed no difference from the other plate in the time of the outburs About 5 per cent of the area of the plate which had been immersed in bay water plus peptone for five days was covered with bacteria when the plate was immersed in the bay; the eighteen day plate was covered to an extent of about 11 per cent. This coating was largely lost within a few days, however. It is noteworthy that, although the time of maximum increase of the diatoms was not affected by the presence of bacteria, larger populations of diatoms eventually developed on the bacteria-coated plates than on the other plates. Algae other than diatoms were not important in this experiment; *Ectocarpus, Enteromorpha, Cladophora, Scytosiphon, Pterosiphonia* and *Lophosiphonia* were noted, but did not appear in quantity until some time after the diatom increase.

The data available from this study are not sufficient to establish a succession within the algal community. Wilson (1925), however, has reported a definite sequence, involving diatoms, hydroids and filamentous algae, on rocks at La Jolla. It is quite possible that careful study over a longer period would reveal a similar situation here.

It appears that the relatively heavy growth of diatoms on the bay water plus peptone plates is correlated with a correspondingly heavy settlement of bryozoans. Whether there is a direct causal relation between diatom growth and bryozoan settlement is uncertain. However, the maximum period of bryozoan settlement never preceded, and usually followed, the period of maximum diatom increase in the experiment of Table IV.

The encrusting bryozoan *Membranipora tuberculata,* which normally inhabits the stipes of kelp, occurred on the experimental plates only on one occasion, and remained only a short time. Most of the colonies fell from the plate within the space of a month. Unlike the other bryozoans, however, this species showed definite preference for the bacteria-coated plate.

These experiments suggest, then, that the important change which occurs on plates favoring bryozoan settlement, is the growth of diatoms. In view of this conclusion and of the fact that the bryozoan community, throughout the course of this study, has been observed to form only on surfaces previously supporting an algal growth, we may say that a definite succession is established.

The observations on the glass plates provide little evidence concerning the other communities, but two instances are worthy of mention. The development of a *Styela* community from a bryozoan community was noted in one instance, on a plate exposed horizontally in March 1943. The algal coat which developed upon exposure was displaced by bryozoans before the end of June. In September, however, specimens of *Styela* which had settled in July had become so large as to dominate the community. The remaining bryozoans gradually lost ground and fell from the plate, leaving *Styela* as the principal organism present. The fact that *Styela* was always found growing out of a substratum of old bryozoan colonies on the floats examined in the course of this study indicates that this sequence probably occurs frequently.

The second instance concerns the formation of a *Balanus* community. The plate concerned was exposed horizontally in August 1943. The algal community formed very rapidly, and in addition, within two weeks, larvae of *Eupomatus*, encrusting bryozoans, *Pecten* and *Balanus* had settled in large numbers. In the ensuing competition for space, the barnacles emerged victorious. In September, there were more than two hundred barnacles on the exposed side of the plate, covering the surface almost completely. An equal number of *Eupomatus* occupied the spaces between the barnacles, but encrusting bryozoans were not abundant. During subsequent months, however, growth of the bryozoans was continuous, and by December, the barnacles were almost completely covered by the rapidly growing bryozoans.

Discussion and Conclusions

In order to make a satisfactory analysis of the phenomena described in the preceding sections, it would be necessary to know (a) the breeding seasons of the organisms involved, (b) the normal duration of life of each of the important organisms, (c) the length of the free-swimming larval period in each case, and (d) the nature of the surfaces to which such free-swimming larvae will attach. We do not have such information in most cases. Nevertheless, it is possible to make some interpretations on the basis of the available information.

It was stated at the outset that the basic problem is that of distinguishing between seasonal progression and true succession. Several examples of progression were noted at Beaufort, N. C. by McDougall (1943). The organisms which settled during the winter were, for the most part, dead or moribund by spring, and were consequently replaced by organisms breeding chiefly in the spring. There is some reason to expect that seasonal progression may be less important in Newport Harbor than at Beaufort. The annual range of monthly mean temperatures at Beaufort is 23° C., from 5.5° in February to 28° in July. The annual range in Newport Harbor is only 5° C., from a low of 14.1° in February to a high of 19.2° in July. The breeding seasons of most of the organisms involved in the sequences described here extend through most of the year. Certainly algae, bryozoans and mussels have been observed to settle during every month of the two years covered by this study.

In the present study, it seems probable that the algal community, and most probably the diatoms comprising the basis of that community, provide favorable conditions for the settlement of bryozoans. Bryozoans settled in quantity only after the development of a fairly vigorous algal community. Moreover, in the experimental test described in Table VI, bryozoan settlement was definitely correlated with the settlement and growth of diatoms. There remains the possibility that some common factor favored settlement of both diatoms and bryozoans, the former remaining "dominant" only until the slower but persistant growth of the latter displaced them. It is difficult to rule out such common factors, but it appears unlikely that chemical alterations in the glass on exposure to sea water are involved. The plates used in this particular experiment had previously been immersed in the bay for two months. They were then scrubbed with a brush in tap water, wrapped in paper and sterilized in an autoclave. The experimental plates were soaked in three liters of solution for several days as noted in the table,

TABLE VII

Weights of organisms removed from glass and aluminum panels, 1943–44
(+ sign indicates organisms present in amounts of less than 1 gram)

Surface:		Glass													Aluminum		G	G	Al
Duration of exposure, weeks:		4			8				12	20					26	28	32	36	
Date of initial exposure:		Mar. 12 '43	Apr. 4 '43	Dec. 20 '43	Mar. 12 '43	May 7 '43	July 5 '43	Dec. 20 '43	Dec. 20 '43	Dec. 20 '43	Jan. 31 '44	Feb. 28 '44	Mar. 27 '44	Apr. 27 '44	Sept. 21 '43	Mar. 28 '44	Jan. 17 '44	Dec. 20 '43	Dec. 14 '43
Total weight of organisms, grams:		20	56	9	23	67	18	12	19	117	204	302	174	157	83	194	193	180	128
Per cent of weight contributed by:																			
Algae, Bacteria, Hydroids, Debris:		100	85	100	87	76	28	100	81	2	1	30	9	29	59	21	18	15	11
Bryozoans	Encrusting forms				+	1	29	+	5	57	54	34	35	41	30	63	27	34	66
	Erect forms			+		9	3	+	+	21	22	11	25	16	2	6	28	17	1
Annelids	Serpulids					+	14	+	+	10	2	1	+	3	6	1	5	8	1
	Others		8		+	1		+	3	1	5	1	1	1	1	1	1	1	2
Crustaceans	Erichthonius		4	+			3	+	11	8	5	21	14	6	1	3	10	19	5
	Balanus						14			+		+	+	+	1	+			1
	Others		3	+	+	1	3	+	+		+	+	+	+	+				+
Mollusks	Pecten				+	10	6	+				+	1		+	+	+	+	+
	Mytilus															3			
	Saxicava				+	+				+	+	+	4	2	+	1	8	4	8
Sponges											5	+			1	+	+	+	+
Ascidians				+	+		+			1	5	1	11	1		1	2	2	5

while the control plates were placed directly in the bay. The same sequence of organisms is apparent on both experimental and control plates, and the periods of time involved are not significantly different.

The data in Table VII are of interest in this connection. It is apparent that the organisms listed fall into four classes: (1) Those which appear in abundance on all plates, but most abundantly on those exposed for the shortest periods (algae, etc.). (2) Those which appear only on plates exposed more than four weeks, and most abundantly on plates exposed twenty weeks or longer (bryozoans, serpulid worms). (3) Those which appear in measurable quantities only on plates exposed twenty weeks or longer, and are not abundant even on plates exposed as long as thirty-six weeks (*Mytilus, Saxicava,* sponges, ascidians). (4) Those which appear irregularly, without relation to the duration of exposure (annelids, *Balanus, Erichthonius* and other crustaceans, *Pecten*).

It is particularly significant that the dominant organisms of the primary communities involved in the sequence described earlier—viz. algae, bryozoans, ascidians and mussels—fall into separate categories on this basis, and that the sequence here is the same as that observed in the sequence of communities. It appears that the settlement of ascidians certainly and mussels probably is favored by the existence of a thriving bryozoan community.

In any event, there is no evidence that seasonal progression is involved to a significant extent in the algae-bryozoan-*Mytilus* sequence. A plate exposed in December went through the same sequence as did one exposed in March or April; the time relations varied, but the sequence did not. And in the absence of a seasonal progression, it is difficult to avoid the conclusion that true succession is involved.

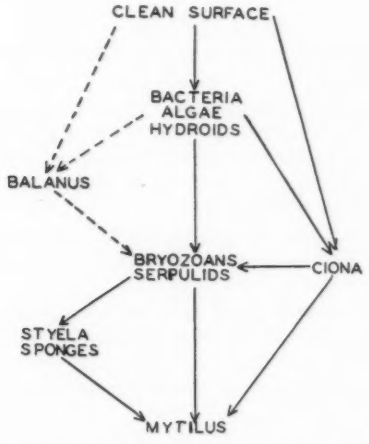

FIGURE 4. Sequence of dominant organisms on surfaces exposed in Newport Harbor.

In many studies of the life histories of sedentary organisms, estimates of the season of settling have been based on the number of new settlers on a plate exposed for a brief period. It is evident from the results reported here that such

estimates may be unreliable if succession is involved in the settlements under consideration. Thus, plates exposed for four weeks or less in Newport Harbor in the winter months would receive few or no settlements of bryozoans, despite the fact that settling larvae are present in the water during these months. It is important, therefore, that studies of this sort take into consideration the question whether succession is occurring.

With the evidence presented in this paper, we can make a number of suggestions as to the possible factors involved in the events described. The sequence is depicted in Figure 4. A newly exposed surface is first colonized by bacteria, algae and, in some seasons, hydroids. The development of these forms provides a favorable surface for establishment of bryozoan colonies, and also for the settlement of serpulid larvae. The vigorous growth of the bryozoans eventually displaces the algae and hydroids. The resulting bryozoan community in turn provides a favorable basis for the attachment of *Mytilus* larvae. The growth of the mussels effectively covers the whole surface of the bryozoan community, the members of which eventually perish from lack of food, oxygen, etc. Seasonal factors, involving the settlement of ascidian or barnacle larvae in tremendous numbers, may introduce variations into this sequence. *Ciona* may sometimes colonize a clean surface, or one covered with algae, to such an extent that the bryozoans are unable to maintain their foothold. *Styela* apparently settles only on bryozoan substrata, but may become established before *Mytilus*, and hence a community dominated by *Styela* may follow the bryozoan stage. Sponges are frequently associated with *Styela*. Both *Ciona* and *Styela* communities are eventually displaced by *Mytilus* which at present represents the climax in the float-bottom associations of Newport Harbor.

Summary

1. The sedentary communities characteristic of float bottoms in Newport Harbor, California, are described.

2. The most important communities at present are dominated, respectively, by algae, bryozoans, *Ciona intestinalis*, *Styela* sp. and *Mytilus* sp.

3. These communities represent stages in an ecological succession.

4. The algal community appears first on freshly exposed surfaces, to be followed usually by a bryozoan community.

5. The bryozoans prominent in the bryozoan community settle more readily on surfaces supporting a vigorous growth of diatoms and other algae than on clean surfaces.

6. The community dominated by *Mytilus* constitutes the climax at present.

7. *Mytilus* has been observed to settle only on surfaces bearing a bryozoan, *Ciona* or *Styela* community.

8. The establishment of *Ciona* or *Styela* communities appears to depend in part on seasonal factors.

Acknowledgment

I should like to express my appreciation of the helpful criticisms and suggestions of Professors W. R. Coe, M. W. Johnson, G. E. MacGinitie and A. C. Redfield. Miss Margaret L. Campbell rendered invaluable technical assistance during a part

of this work. I am indebted to Dr. Raymond C. Osburn for identification of the bryozoans.

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A COMPARISON OF THE EFFECTS OF CYANIDE AND AZIDE ON THE DEVELOPMENT OF FROGS' EGGS [1]

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Loeb's (1895) observations that the eggs of *Fundulus heteroclitus* are capable of considerable development under anaerobic conditions has since been extended to various amphibian embryos. Brachet (1934), in confirming the possibility of anaerobic development for *Rana temporaria* eggs, reported also that cyanide is similar to anaerobiosis in its effects on embryogenesis. Eggs placed in cyanide immediately after fertilization were arrested in the late blastula, but those placed in cyanide after gastrulation had begun would continue to the formation of a complete blastopore. Later stages were increasingly sensitive to cyanide. Although it has generally been thought that the arrests of development caused by cyanide are due to inhibition of the cytochrome oxidase of the Warburg-Keilin system (Keilin, 1933), it might be inferred from the recent work of Holtfreter (1943) that the repressive effects of cyanide solutions result merely from their alkalinity. It will be shown in this paper however that only post-mortem effects are influenced by the pH of the cyanide solution, the actual stoppage resulting from the presence of the toxic radical itself.

In 1936 Keilin reported in detail on another specific inhibitor of cytochrome oxidase, sodium azide (NaN_3). On the basis of these experiments NaN_3 and NaCN have in some cases been used interchangeably. Philips (1940), in comparing the developmental sensitivities to anaerobiosis of pelagic and non-pelagic fish eggs, employed both NaCN and NaN_3. He found that Fundulus eggs before the end of gastrulation are capable of extensive development in concentrations of both cyanide and azide which completely and almost immediately inhibit pelagic eggs. Except for the higher concentrations required in the case of NaN_3 he could demonstrate no difference between the effects of the two reagents. Recently Barnes (1944) tested the same reagents on the development of *Rana pipiens*. The results with cyanide confirmed the earlier observations of Brachet (1934). While no detailed data are given, the effects of azide were apparently found to completely parallel those of cyanide, for Barnes states: "Eggs exposed to M/100 NaN_3 at pH 7.0 are able to develop to the gastrula stage. Gastrulation never occurs in the presence of azide." Lower concentrations (M/1000) did not stop gastrulation although the eggs developed at a slower rate.

The present authors (Moog and Spiegelman, 1942), while investigating the relation between regeneration and metabolic activity, demonstrated a specific difference between the effects of azide and cyanide on hydranth reconstitution in Tubularia. Azide could inhibit regeneration at concentrations which did not sensibly

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affect respiration whereas cyanide caused parallel depressions in rates of regeneration and of respiration. Subsequent analysis (Spiegelman and Moog, 1944) of the differential effects of these two agents on the mass and time of appearance of the new hydranth emphasized the difference in their activities.

In the fall of 1941 the authors undertook a comparison of the effects of NaCN and NaN_3 on the development of *Rana pipiens*.[3] The results obtained disagree in certain respects with those reported by Barnes (1944). Azide was found to be completely effective in stopping morphogenesis at all stages of development, including those between fertilization and gastrulation which are not inhibited by cyanide. In an effort to discover the cause for the disagreement these experiments were repeated recently under conditions closer to those employed by Barnes. Our earlier results were confirmed and the discrepancy remains unresolved. No direct comparison with the findings of Philips (1943) is possible, not only because of the difference in material but also because the highest concentration he employed was below the one we found to give consistent inhibitions.

The results will be detailed and the difference obtained between the effects of azide and cyanide will be discussed in the light of recent findings on azide inhibitions of anaerobic synthetic processes.

General Methods and Materials

Eggs of *Rana pipiens*, obtained by injection of pituitary glands, were expressed and artificially fertilized. After swelling of the jelly the eggs were cut up into small groups in 10 per cent Ringers solution adjusted to the desired pH with phosphate buffer. Stages were determined according to the schedule of Pollister and Moore (1937) and are so numbered in the present paper. The eggs were stripped from the jelly with fine forceps before being immersed in the experimental solutions.

All hydrogen ion concentrations were determined with a glass electrode after the reagents were added. Where temperature control is indicated the designated temperature was held within $\pm 0.2°$ C. Other experimental details will be found in the appropriate places of the text.

Experimental Results

The effects of azide and cyanide on development

Keilin (1936) as well as subsequent investigators demonstrated the critical influence of pH on the effectiveness of azide as a respiratory inhibitor. Using the isolated Warburg-Keilin system as well as yeast cells Keilin obtained maximal effects at about pH 6.3 when the azide was used in concentrations of 0.001 and 0.002 molar. In the experiments to be described in the present section azide solutions were adjusted to pH 6.6. The concentration chosen for study was 0.005 molar, since parallel experiments on the effects on respiration (see Spiegelman and Steinbach, 1945) indicated maximal effects at this concentration on respiratory rate. The same can be said for development, for 0.005 M azide yields completely effective inhibition. All controls for the azide experiments were similarly adjusted

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to pH 6.6. In the case of cyanide both experimentals and controls were run at pH 8.4. The controls at pH 8.4 did not differ detectably in rate of development from those at pH 6.6. Every experimental set had its own control and both were thus handled exactly the same number of times and in the same fashion. This avoided the relatively more frequent handling and examination of the controls which would have been necessary if one set of eggs were the controls for a larger number of experimentals. For convenience in observations all of the present experiments were done at 15.2° C. in a cold room. To avoid the accelerating and decelerating effects of changing temperatures during development (see Ryan, 1943) the eggs were kept at 15.2° C. in 10 per cent Ringers until they reached the stage it was desired to test. They were then transferred to the approximate solutions previously brought to the same temperature. The cyanide solutions were freshly prepared and renewed every 12 hours during the course of an experiment; the experimental solutions were kept in stacked fingerbowls, with an empty bowl covering the top member of the stack.

For the purposes of comparison with the azide experiments, the results with cyanide in the early stages are reproduced in Table I. They do not differ in essentials from those reported by Brachet (1934). Eggs placed in cyanide early in development, although delayed as compared with controls, continue to develop up to gastrulation. The later the stage at which they are subjected to cyanide the closer is the approach to gastrulation; they do not however actually begin to gastrulate. Eggs in early stage 9 will continue to segment until the cells at the vegetal pole are quite minute but will evidence no signs of dorsal blastopore lip formation. If however the invagination has already started cyanide will not immediately stop it and the eggs may proceed to the formation of a complete blastopore before ceasing activity. Later stages become increasingly sensitive to cyanide.

TABLE I

The effects of cyanide on development at pH 8.4 at 15.2° C. These experiments were done in 1941–2 on material obtained from Vermont. The numbers represent the developmental stages as described under Methods.

Stage at start of experiment	Solution	Hours after immersion											No. of eggs
		4	8	12	18	24	35	45	55	75	95	120	
Uncleaved	0.001 M	2	3		6	8	8	8	8				210
Uncleaved	Control	3	3		8	8	10	11	12				210
3	0.001		4		6	7	8	8	9	9	9		200
3	Control		6		8	9	10	11	12		16		200
6	0.001		7			8	8	8	8	9	9	9	160
6	Control		8			10	11	12	12	13	16	18	160
9	0.001		9		9		9	9					200
9	Control		10		11		12	13		16			200
10	0.001			10		10		11		12		12	200
10	Control			11		12		14		16		18	200

The results obtained with azide are summarized in Table II. It is immediately evident that all stages are azide sensitive, including the early ones which are not effectively inhibited by cyanide. It might be noted that under these experimental conditions the cessation of developmental activity on immersion in azide solution is, as far as can be determined, abrupt and immediate. This was easily ascertained

TABLE II

The effect of azide on development at pH 6.6 at 15.2° C.; 1941–2, material from Vermont. The numbers represent the developmental stages as described under Methods

Stage at beginning	Solution	Hours after immersion											No. of eggs
		4	8	12	18	24	35	45	55	75	95	120	
Uncleaved	0.005 M	1		1			1	1	1	1	1	1	100
Uncleaved	Control	3		5			10	11	12	14	16	18	100
6	0.005 M		6			6	6	6	6	6	6	6	150
6	Control		7			9	10	11	12	14	16	18	140
7	0.005 M		7	7		7		7	7	7	7	7	90
7	Control		9	10		11		12	14	16	17	18	90
9	0.005 M	9			9			9		9	9	9	110
9	Control	10			11			13		16	17	18	110
10	0.005 M			10			10			10	10		160
10	Control			11			13			17	18		160
11	0.005 M		11				11		11		11		85
11	Control		12				14		16		18		85
12	0.005 M					12			12				105
12	Control					14			16				105
13	0.005 M		13			13	13		13				120
13	Control		14			16	17		18				120
14	0.005 M				14		14	14			14		90
14	Control				16		17	18			19		90
16	0.005 M			16			16		16	16			110
16	Control			17			18		18	19			110
17	0.005				17	17	17	17	17				60
17	Control				18	18	18	18	19				60

in the early cleavage stages since no further cleavage was observed. Although the observations are more difficult in the later stages, careful examination failed to reveal any development subsequent to treatment with azide. If the eggs are removed within 30 minutes after being placed in the azide solution and thoroughly washed they can proceed with their development.

Barnes' (1944) experiments with azide were done at higher temperatures, concentrations, and pH than those described above. Accordingly when the azide ex-

periments were repeated they were done at room temperature (ca. 25° C.), at pH 7.4 and 8.3 (i.e. with and without added hydrochloric acid), and with concentrations up to 0.01 M. The results of these experiments are given in Table III. At both hydrogen ion concentrations, 0.01 M azide caused immediate arrest in all pregastrular stages. The 0.005 M concentration used in the early experiments was retested under these conditions and found to give exactly the same results as previously obtained. Controls kept in Ringers buffered at the experimental pH developed normally in all cases, and are not reported in the table.

TABLE III

The effect of azide on development at 25° C.; 1944–5, material from Wisconsin

Stage at immersion	Conc. (Molar)	pH	Stage at arrest
1	0.001	7.4	9*
1	0.001	8.3	9*
1	0.005	7.4	1
1	0.005	8.3	1
1	0.01	7.4	1
1	0.01	7.4	1
1	0.01	8.3	1
7	0.005	7.4	7
7	0.01	7.4	7
9	0.005	7.4	9
9	0.005	7.4	9
11+	0.01	8.3	12−
13	0.01	8.3	13

* There was no delay in reaching this stage.

It is clear that we can offer no support to Barnes' statement that at the concentration and pH she employed, azide, like cyanide, permits eggs to develop to gastrulation.

The effect of NaCN on development at different pH values

Holtfreter (1943) presented evidence showing that the disruptive effects of strong cyanide solutions (0.1 M to 0.0015 M) can be imitated by potassium hydroxide solutions of equal pH. Although the author did not specifically claim that the oxidation-repressing effects of cyanide are to be regarded as completely irrelevant to its influence on development, it nevertheless seemed advisable to us to clarify the points which were left in an indecisive state by Holtfreter's work. This we did, in our 1944–1945 series of experiments, both by comparing the effects of NaCN solutions brought to pH 7.2 with HCl with those at pH 9.6–9.8, and by determining the effects of solutions of either NaOH or KOH at pH 9.8. The tests were made at about 25°; the cyanide solutions were changed three times daily, the hydroxide solutions once daily.

The results of the NaCN tests completely confirmed our earlier findings (Table IV). The stage in which development was stopped, and the speed with which that stage was reached, was in all cases the same in solutions of equal concentration at both low and high pH. Only after the egg had been in an arrested stage for

more than 12 hours did a difference between the two pH's become evident. At high alkalinity the pigment became streaked, the surface disintegrated, and the egg was in the majority of cases reduced to a loose, fuzzy mass of cells within 36 hours; at low alkalinity the surface was only moderately eroded after 72 hours.

TABLE IV

The effects of NaCN at pH 7.2 and 9.8; 1944–5, material from Wisconsin

Stage at immersion	Conc. (Molar)	pH 7.2		pH 9.6–9.8	
		Stage at arrest	Later effects	Stage at arrest	Later effects
1 1	0.003 0.006	Not tested Not tested		7 7	Egg swollen and surface severely depigmented after 36 hrs.
7 7	0.003 0.006	9 9	Marked depigmentation after 40 hrs.	9 9	Depigmentation after 20 hrs., surface disintegrated after 36 hrs.
9 9	0.003 0.006	11 11	Blastopore lip disappeared within 20 hrs. after forming	11 11	Blastopore lip also disappeared. Surface completely disintegrated after 24 hrs.
1	0.004	7	Surface became mottled but did not disintegrate within 72 hrs.	7	Complete disintegration within 24 hrs.
1	0.004	7	Egg swelled to twice its normal diameter but did not disintegrate within 96 hrs.	7	Complete disintegration within 24 hrs.
8	0.004	9	Surface became mottled and egg swelled somewhat, but did not disintegrate within 88 hrs.	9	Surface became mottled within 24 hrs., complete disintegration within 38 hrs.

The studies with hydroxides revealed that *Rana pipiens* eggs can develop from fertilization to the stage of tail-fin circulation (stage 22, at which they were discarded) at pH 9.8 (i.e., 2.5×10^{-4} M). Stage 22 was also achieved uneventfully if the eggs were immersed in the hydroxide solutions at the stage of the morula (S7), late blastula (S9), mid-gastrula (S11), neurula (S14), muscular movement (S18); in the last two cases the vitelline membrane was removed before the embryos were placed in the alkali solutions. In complete contradiction to Holtfreter's finding that eggs disintegrate in the morula stage in KOH solutions of pH 9.0 to 9.4, we did not observe either retardation or abnormality of development. In three experiments with NaOH and two with KOH, we obtained identical results. Thus we may conclude that the suppressive action of NaCN (or KCN) on living egg is due to the poisonous effect of the CN component.

Discussion

Both azide and cyanide are effective inhibitors of respiration in the early as well as in the later stages of development (Barnes, 1944; Spiegelman and Steinbach, 1945). The fact that cyanide cannot inhibit at any stage before gastrulation whereas azide can inhibit at all stages, cannot be explained on a respiratory basis. This is even more pointedly demonstrated by the capacity of eggs to develop to gastrulation under anaerobic conditions. The ability of cyanide to depress respiratory rates at all stages clearly proves that it gets into the cells of the early embryos, and consequently a difference in permeability cannot be invoked to explain the difference between the effects of azide and cyanide on development. It is clear from these experiments that, at least in the early stages, NaN_3 is inhibiting some cyanide-insensitive process necessary for development.

Recent work has served to question conclusions drawn from Keilin's earlier experiments that azide and cyanide are essentially equivalent inhibitors of the Warburg-Keilin system. Stannard (1939) showed that cyanide inhibited the respiration of both resting and active muscle while azide affected active muscle only. Armstrong and Fisher (1940) demonstrated that azide and cyanide behave differently in inhibiting the enzymes controlling the frequency of the embryonic fish heart-beat. Differences in cyanide and azide inhibitions of tissue respiration led Korr (1941) to postulate the existence of different pathways of respiration in resting and stimulated tissues. Ball (1942) suggested different oxidation-reduction potentials for the Atmungsferment-cyanide and Atmungsferment-azide compounds as an explanation of the different effects of the two inhibitors. Winzler (1943), after subjecting the kinetics of the respiratory inhibition by cyanide and azide in yeast to a careful examination, came to the conclusion that cyanide inhibited yeast respiration by three different pathways: (1) by combining with oxidized Atmungsferment; (2) by increasing the apparent KO_2 of reduced Atmungsferment; and finally (3) by combining with the enzyme which controls the rate-limiting step of the rate of respiration. Azide on the other hand exhibited only one type of inhibition, namely, combination with oxidized Atmungsferment.

Aside from these studies on respiration, others have been made on assimilatory activity of microorganisms. Barker (1936) and Giesberger (1936) showed that suspensions of bacteria could under certain circumstances synthesize carbohydrate from various substrates. Clifton (1937) studied the effect of azide on these syntheses and found them to be completely inhibited. In the presence of azide external substrate was completely oxidized. Clifton and Logan (1939) extended these findings and showed that it was possible to differentially inhibit assimilatory processes with both NaN_3 and 2, 4-dinitrophenol. Winzler (1940), working with acetate assimilation in yeast, showed that low concentrations of azide, cyanide, or 2, 4-dinitrophenol prevented assimilation. Azide was also shown by Winzler (1944) to prevent the anaerobic assimilation of glucose by yeast without interfering with its fermentation. Winzler, Burk, and du Vigneaud (1944) found that azide in concentrations of 10^{-4} and 10^{-3} molar inhibits completely the anaerobic assimilation of ammonia.

These experiments show that azide, and in certain instances cyanide, can inhibit synthetic processes which are essentially anaerobic in nature and not connected with the Warburg-Keilin system. It seems most probable that it is this

sort of inhibitory activity which is involved in the ability of azide to stop embryonic development. Unfortunately, with the exception of Winzler's (1940) study of acetate assimilation no detailed comparison between the effects of azide and cyanide on synthetic processes has been published. In view of the results reported in the present paper one would venture to predict that such differences will be discovered. It may be noted that one such difference has been found in the case of adaptive enzyme formation in yeast, which is azide sensitive but is not inhibited by cyanide (Spiegelman, 1945). A suggestive finding has been reported recently by Meyerhof (1945), who prepared a solution of adenylpyrophosphatase from yeast by supersonic vibration and found it insensitive to cyanide but highly sensitive to azide. This enzyme, involved as it is in transphosphorylation, might conceivably be a part of the azide sensitive anaerobic synthetic processes.

Summary

Previous observations that amphibian eggs can develop up to the beginning of gastrulation in cyanide solutions have been confirmed on eggs of *Rana pipiens*. The effect of cyanide is independent of pH, and eggs can develop into tadpoles in 2.5×10^{-4} molar NaOH or KOH solutions at pH 9.8.

Azide has been found to arrest development immediately in all stages from fertilization to tail-bud formation. The effect is the same from pH 6.6 to pH 8.3.

These differences are discussed in the light of recent studies on the effects of azide and cyanide on respiratory, assimilatory, and phosphorylative processes.

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